CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761177Orig1s000

NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	BLA 761177
Regulatory Pathway:	351(a)
Supporting document/s:	0001
Applicant's letter date:	June 25th 2020
CDER stamp date:	June 25th 2020
Product:	Lonapegsomatropin (ACP-011)
Indication:	Growth Hormone Deficiency (Children)
Applicant:	Ascendis Pharma A/S
Review Division:	DGE/OCHEN
Reviewer:	Jeffrey Quinn, PhD
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Review Completion Date:	February 25th 2021

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1 Executive Summary

1.1 Introduction

Ascendis Pharma A/S submitted a Biologics License Application (BLA 761177; 351a) for lonapegsomatropin, a long-acting (pegylated) human growth hormone (hGH) pro-drug intended to release somatropin resulting in the same mode of action and distribution as daily hGH, but via a once-weekly subcutaneous injection for the treatment of GH deficiency in children.

Pegylation of hGH is intended to reduce the elimination of lonapegsomatropin (hGH bound to carrier) and facilitate a prolongation of the plasma half-life. Lonapegsomatropin itself exhibits reduced binding to the hGH receptor and minimal in vitro activity compared to unconjugated hGH. The hGH released from lonapegsomatropin following hydrolysis is fully active (as unmodified hGH) and is intended to achieve the same tissue distribution and effects of daily hGH replacement therapy and result in IGF-1 levels within the physiologic range. The nonclinical data indicates that the pharmacodynamics and biological activity of the hGH released from lonapegsomatropin are comparable to those of other hGH products.

The nonclinical safety evaluation of lonapegsomatropin and the products of its autocleavage (hGH, (b) (4) (TransCon linker leaving group released during autocleavage), mPEG-linker (b) (4) TransCon linker attached to mPEG) and mPEG (methoxypolyethylene glycol carrier molecule) included genotoxicity, safety pharmacology, local tolerance, reproductive toxicity, and repeat-dose toxicity studies in rats (≤ 26-weeks) and juvenile monkeys (≤ 52 weeks). These studies yielded findings consistent with hGH activity and with cellular uptake of the released mPEG moiety at lonapegsomatropin dose levels ≤ 4.8 mg/kg/week (20-fold above the expected clinical therapeutic dose level of 0.24 mg hGH/kg/week).

Independent reviews conducted by the Division of Neurology and the Division of General Endocrinology of the nonclinical safety assessment of mPEG accumulation observed in the choroid plexus of rats and monkeys (26/52-Week, pivotal toxicity studies) concluded that there is minimal risk of adverse neurological effects at the clinical therapeutic dose level of 0.24 mg hGH/kg/week in GHD children.

Drug-related effects noted during the pivotal toxicity studies were consistent with the known effects of administering hGH to SD rats and cynomolgus monkeys. Hence, the high dose (4.8 mg hGH/kg/week) was considered the most clinically relevant NOAEL. Exposure margins between the nonclinical NOAEL and the steady state exposure levels at clinical exposure (0.24 mg hGH/kg/Week) are depicted below.

Comparison of Exposure at the NOAEL for the Pivotal Toxicity Studies in SD Rats and Cynomolgus Monkeys with the MRHD in GHD Children

Analyte	Species/ Duration	Mean AUC_{0-168} [μg.hr/mL]	Mean C_{max} [ng/mL]	MoE[a] AUC	MoE[a] C_{max}
hGH	Rat/26 Week	-	20	-	1x
	Monkey/52 Week	26.1	320	38x	17x
	GHD Children	0.68	18.5	-	-
mPEG	Rat/26 Week	7455	55000	4x	4x
	Monkey/52 Week	58900	425000	34x	17x
	GHD Children	1740	13100	-	-
Lonapegsomatropin	Rat/26 Week	-	469	-	0.4x
	Monkey/52 Week	3850	37300	52x	30x
	GHD Children	74	1230	-	-

[a] Multiple of Exposure (MoE) was calculated as ratio of serum PK parameters at the no observed adverse effect level (NOAEL) in the pivotal toxicity studies and the 0.24 mg hGH/kg/week clinical dose in GHD children.

1.2 Brief Discussion of Nonclinical Findings

Toxicology data used to support approval of lonapegsomatropin was derived from the pivotal toxicity studies conducted in SD rats and cynomolgus monkeys. A primary finding was the presence of mPEG in injection site tissues and in some cell types of the choroid plexus, notably the epithelium, macrophages, and specialized glia. An extended period of recovery (i.e., non-dosing period) demonstrated clearance of mPEG from the tissue sites with the exception of the choroid plexus epithelium, which retained a minimal to mild level of mPEG cellular vacuolation. The nonclinical safety assessment indicates that the retained presence of mPEG and cellular vacuolation of the choroid plexus epithelium in rats and monkeys represents minimal-to-no risk to children with GHD based on the following considerations:

- Vacuolation of the choroid plexus epithelium was not observed histologically in cynomolgus monkeys after 52 weeks dosing at an exposure 2-fold higher than the clinical dose, and higher exposures of 9-10-fold the clinical dose were necessary to result in minimal epithelial vacuolation.
- The presence of mPEG detected by immunohistochemical staining and epithelial vacuolation observed in the choroid plexus was not associated with histological evidence of structural changes to tissue architecture, degeneration, necrosis, or inflammation, and no clinical signs of neurotoxicity were observed (e.g. tremors, convulsions, reactivity to handling or unusual behavior).
- mPEG was not detected in the cerebral spinal fluid (CSF) above the lower limit of quantification (LLOQ, 500 ng/mL) in either SD rats or cynomolgus monkeys administered lonapegsomatropin. In addition, hGH and mPEG were not detected in brain tissue including areas associated with high levels of growth hormone receptor (GHR) expression.

The mPEG weekly dose level (total mPEG load) administered to children with GHD at the clinical dose was 8-fold below the theoretical threshold (3.7 mg PEG (40 kDa) /kg/QW or 0.4 μmol PEG/kg/month) recommended by the Committee for Medicinal Products for Human Use (CHMP 2012) for choroid plexus epithelial cell vacuolation[1]. In addition, systemic mPEG concentrations at steady state (15 μg/mL) in children with GHD administered lonapegsomatropin were 7-fold below the mPEG exposure vacuolation threshold (100 μg/mL) defined by Jacobsen/Bjørnsdottir (FDA Briefing Document[2] and BioSafe EU, 2017[3]).

The toxicology results with lonapegsomatropin appear consistent with the view of the Society of Toxicologic Pathology (STP) Working Group publication (Irizarry et al. 2018) that accumulation of mPEG in the choroid plexus epithelium of animals administered repeat-doses of PEGylated products appears to be an adaptive, non-adverse finding, based on the lack of any signs of tissue degeneration or dysfunction, in multiple published studies[4].

Nonclinical Exposure Margins at LOEL for mPEG Accumulation in the Choroid Plexus

Nonclinical Finding	Reversible?	Species/ Duration	LOEL Dose mPEG (Cmax/AUC)	MoE[a] (Cmax/AUC)
Choroid Plexus Epithelial Cell Vacuolation (H&E)	No	Monkey (52-Week)	1.6 mg hGH/Wk (122 μg/mL) (17600 μg.hr/mL)	9x/10x
Choroid Plexus Macrophage Vacuolation (H&E)	Yes	Monkey (52-Week)		
Choroid Plexus mPEG Staining - Cellular Vacuolation (IHC)	Partial	Monkey (52-Week)	0.4 mg hGH/Wk (24.6 μg/mL) (3330 μg.hr/mL)	2x/2x
	No	Rat (26-Week)	1.2 mg hGH/Wk (15.7 μg/mL) (2200 μg.hr/mL)	1x/1x

[a] Multiple of Exposure (MoE) was calculated as ratio of serum mPEG exposures at the lowest observed effect level (LOEL) in the pivotal toxicity studies and the 0.24 mg hGH/kg/week clinical dose (13.1 μg/mL, C_{max} and 1740 μg.hr/mL, $AUC_{0\text{-}168hrs}$) in GHD children.

[1] CHMP Safety Working Party's response to the PDCO regarding the use of PEGylated drug products in the pediatric population, November 16, 2012.
[2] Jacobsen H, Bjørnsdottir I. FDA Briefing Document Prepared for the Blood Products Advisory Committee April 4, 2017, BLA 125611, Coagulation Factor IX (Recombinant), GlycoPEGylated N9-GP Novo Nordisk, Inc. 2017.
[3] Jacobsen H, Bjørnsdottir I. Learnings from recent regulatory submission with 40 kDa PEGylated coagulation factor IX (N9-GP) PK and Safety. In: Presented at: BioSafe European Annual General Membership Meeting, November 14-15, 2017.
[4] Society of Toxicologic Pathology (STP) Working Group commissioned by the Scientific and Regulatory Policy Committee (SRPC) of the Society of Toxicologic Pathology, Irizarry et al., 2018, Toxicologic Pathology 46(6):616-635.

1.3 Recommendations

1.3.1 Approvability

There are no deficiencies in the nonclinical data that would preclude approval of BLA 761177.

1.3.2 Additional Nonclinical Recommendations

No additional nonclinical studies are recommended.

1.3.3 Labeling

8.1 Pregnancy

Risk Summary

There are no available data on lonapegsomatropin use in pregnant women to inform a drug associated risk of adverse developmental outcomes.

In animal reproduction studies, there was no evidence of embryo-fetal or neonatal harm when pregnant rats were administered subcutaneous lonapegsomatropin at doses up to 13-fold the clinical dose of 0.24 mg hGH/kg/week *(see Animal Data*).

The estimated background risk of birth defects and miscarriages for the indicated population is unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriages in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

Data
Animal Data

No embryonic or fetal development toxicities occurred in rats administered subcutaneous lonapegsomatropin at doses up to 13-fold the clinical dose of 0.24 mg hGH/kg/week.

In a peri- and post-natal developmental study in rats, there were no adverse effects on the pregnant/lactating female or on development of the conceptus and the offspring following exposure of the female from implantation through weaning to doses of a structurally related pegylated somatropin up to 13-fold the clinical dose of 0.24 mg hGH/kg/week.

13.1 Carcinogenicity, Mutagenesis, Impairment of Fertility

Carcinogenicity studies have not been conducted with (b) (4)

(b) (4) was not mutagenic in the Ames test, in the human chromosomal aberration assay or in the rat bone marrow micronucleus test.

(b) (4)

In an animal fertility study, (b) (4) was administered via subcutaneous injection to male and female rats before cohabitation, through mating to implantation. (b) (4) did not affect fertility or early embryo-fetal development at doses up to 20-fold the clinical dose of 0.24 mg hGH/kg/week.

2 Drug Information

2.1 Drug: Lonapegsomatropin (Skytrofa)

CAS Registry Number

1934255-39-6

Code/Generic Name/Nonproprietary Name

ACP-011/TransCon PEG40 hGH/Lonapegsomatropin-tcgd

Chemical Name

Poly(oxy-1,2-ethanediyl), α,α',α'',α'''-[[[5-[4-[[[[3 (dimethylamino) propyl] methylamino] carbonyl]oxy]phenyl]-5-(carboxyoxy)-1-oxopentyl]imino]bis[6,1-hexanediylthio(2,5-dioxo-3,1-pyrrolidinediyl)(1-oxo-3,1-propanediyl) imino-3,1-propanediyloxy-3,1,2-propanetriyl]] tetrakis[ω-methoxy-, amide with human somatotropin

Somatropin (growth hormone) (Homo sapiens) statistically mono substituted on N6 of lysine residues with the radical: {(1RS)-5-[bis(6-{[(3RS)-1-{3-[(3-{(2RS)-2,3-Is methoxypoly (ethyleneoxy)] propoxy}propyl)amino]-3- oxopropyl}-2,5-dioxopyrrolidin-3-yl]sulfanyl}hexyl)amino]-1-[4-({[3-(dimethylamino)propyl]methylcarbamoyl}oxy)phenyl]-5-oxopentoxy}carbonyl

Molecular Formula/Molecular Weight

The molecular formula of lonapegsomatropin can be expressed as the sum of:

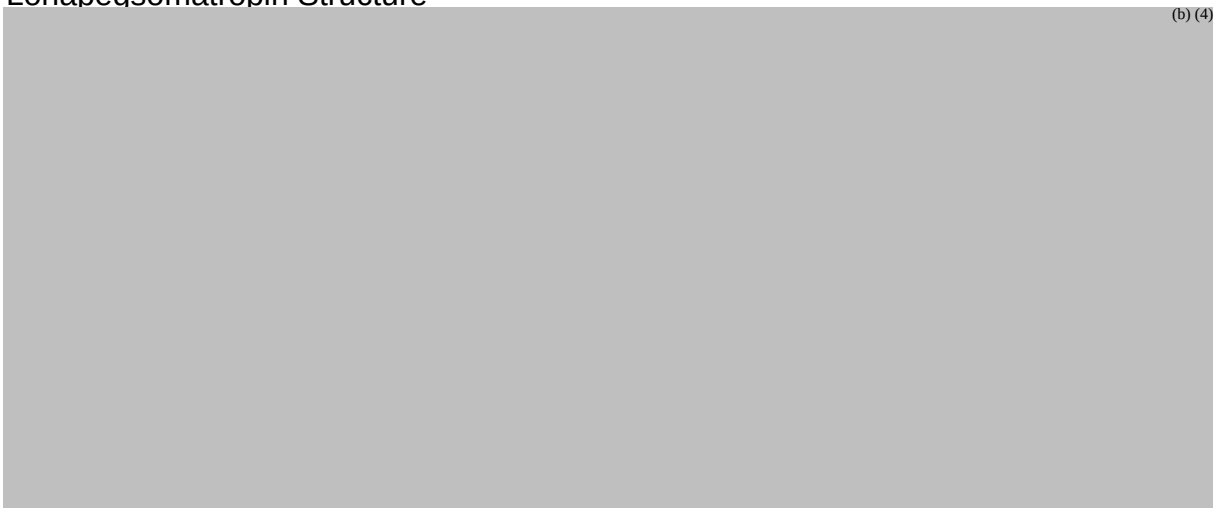
- The molecular formula for hGH - $C_{990}H_{1528}N_{262}O_{300}S_7$,
- The molecular formula for the non-ethylene-glycol-unit part of the mPEG-Linker – $C_{61}H_{100}N_7O_{17}S_2$, and
- The molecular formula for the ethylene-glycol-unit part of the mPEG-Linker – $C_{8c}H_{16c}O_{4c}$, where c is the average number of ethylene glycol units in each of the four PEG-chains.

The average molecular weight of lonapegsomatropin is approximately 63 kDa.

Structure or Biochemical Description

Lonapegsomatropin is produced through chemical conjugation of human growth hormone (hGH) Intermediate (recombinant; MW approximately 22 kDa) to mPEG-Linker (MW approximately 41 kDa). The mPEG-Linker is generated by (b) (4)

Lonapegsomatropin Structure



Pharmacologic Class

Human Growth Hormone (Long-Acting, Pegylated)

Planned Clinical Route of Administration

Subcutaneous Injection

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND (b) (4)
IND 126053 (ACP-011)

2.3 Drug Formulation

Lonapegsomatropin is presented in a dual-chamber cartridge (DCC) containing a lyophilized powder in chamber 1 and a diluent for reconstitution in chamber 2.

Following reconstitution, utilizing a proprietary, non-integral GH auto-injector, the lonapegsomatropin drug product will be presented as a single-use, sterile solution for subcutaneous injection to be automatically dosed by the GH Auto-Injector as a single-dose empty-all concept.

The lonapegsomatropin drug product (in the DCCs) and the GH Auto-Injector is classified as a cross-labeled, non-integral combination product as the cartridges and the auto-injector are packaged separately but labeled for use only with each other.

Lonapegsomatropin is supplied in nine strengths to accommodate dosing of the intended patient group.

Composition of Lonapegsomatropin Reconstituted Solution

Name of Ingredient	Strength (b) (4) Concentration	Function	Quality Standard
Lonapegsomatropin (mg hGH/mL)	(b) (4)	Prodrug form of hGH	Manufacturer's standard
Succinic acid (mg/mL)		(b) (4)	USP-NF
Trehalose dihydrate (mg/mL)			Ph. Eur, USP-NF
Tromethamine	q.s. to pH 5.0		Ph. Eur, USP-NF
Water for Injection	(b) (4)		Ph. Eur, USP-NF

[a] Corresponding to (b) (4) mM

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

A QSAR analysis performed by CDER staff for the (b) (4) indicated that the (b) (4) degradation component, (b) (4) (b) (4) was predicted to be positive for genotoxicity. (b) (4) was negative for mutagenicity and clastogenicity in an Ames assay using hydrolyzed (b) (4) (b) (4) and an in vivo clastogenicity assay (micronucleus test) in rats.

2.6 Proposed Clinical Population and Dosing Regimen

Lonapegsomatropin is indicated for (b) (4)

Lonapegsomatropin will be administered subcutaneously into the abdomen, buttock or thigh with regular rotation of the injection sites. The recommended starting dose is 0.24 mg hGH/kg body weight once weekly. The starting dose will be individualized based on etiology of GHD, treatment goals and the expected sensitivity to therapy.

2.7 Regulatory Background

The predecessor of lonapegsomatropin (ACP-001) was used in early development and employed the identical recombinant hGH and TransCon linker moieties (mPEG-linker and (b) (4) and varied from lonapegsomatropin (b) (4) (b) (4).

Based upon the overall similarity of the kinetics of the released hGH and subsequent insulin-like growth factor 1 (IGF-1) response as well as the associated efficacy and safety profiles observed in nonclinical studies and in a clinical phase 1 trial, data obtained for ACP-001 was considered directly applicable to lonapegsomatropin.

3 Studies Submitted

3.1 Studies Reviewed (Previously Reviewed Under IND 126053)

Safety Pharmacology

Type of Study	Species and Strain or Test System	Duration and Administration Schedule	Doses (mg hGH/kg)	GLP Compliance	Study Number	NOAEL (mg hGH/kg)
Safety Pharmacology						
hERG assay (partly cleaved ACP-001)[a]	Transfected CHO-K1 cell line	NA	5.0 mg hGH/mL	Yes	790652	NA
Neurobehavioral evaluation	Male Sprague Dawley rats	Single administration	0 (vehicle), 0.75, 1.5 and 3.0	Yes	1704-027	3.0
Respiratory evaluation	Male Sprague Dawley rats	Single administration	0 (vehicle), 0.75, 1.5 and 3.0	Yes	1704-028	3.0

ADME/Pharmacokinetics/Pharmacodynamics

Type of Study	Species and Strain or Test System	GLP compliance	Study Number
Analytical comparability of lonapegsomatropin and evaluation of the chemical integrity of released hGH Proliferation activity to determine the potency of hGH released from lonapegsomatropin[a]	In vitro In vitro, rat lymphoma cell line, Nb2-11	No	TH1411041
An assessment of the binding of lonapegsomatropin and hGH to the hGH receptor (hGHR) by surface plasmon resonance (b) (4)[a]	In vitro, soluble hGH receptor	No	OX-15/099-050
Synthesis, purification and determination of the biological potency of permanently PEGylated hGH[a]	In vitro, rat lymphoma cell line, Nb2-11	No	TK1602251
10-day pharmacology study measuring weight gain after administrations of ACP-001 every 3rd day compared to daily administrations of somatropin[b]	Hypophysectomized Sprague Dawley Rats	No	70474/70516/70517
4-week repeat-dose study measuring IGF-1 stimulation after weekly treatment of ACP-001 compared to daily treatment of somatropin (b) (4)[b]	Adult cynomolgus monkeys	No	1704-001, 1704-002

[a] Studies included ACP-001 as a comparator
[b] Performed with ACP-001 but considered applicable to lonapegsomatropin

Toxicology

Type of Study	Species and Strain or Test System	Duration and Administration Schedule	Doses (mg hGH/kg)	GLP Compliance	Study Number	NOAEL (mg hGH/kg)
Repeat-dose Toxicity Studies						
4-week (5 doses) repeat-dose toxicity study — *Bone marrow micronucleus evaluation included*	Male and female Sprague Dawley rats (Rat bone marrow [male])	Weekly dosing for 4 weeks followed by 2-week recovery period	0 (vehicle), 0.75, 1.5 and 3.0	Yes	1704-033	3.0
27-week repeat-dose toxicity study	Male and female Sprague Dawley rats	Weekly dosing for 27 weeks followed by 27-week recovery period	0 (vehicle), 1.2, 2.4 and 4.8	Yes	1704-037	4.8
4-week repeat-dose investigative toxicity study — *Cardiovascular functional evaluation included*	Male and female adult cynomolgus monkeys	Weekly dosing for 4 weeks	3.0	No	1704-019	3.0
4-week repeat-dose toxicity study — *Cardiovascular functional evaluation included*	Male and female adult cynomolgus monkeys	Weekly dosing for 4 weeks followed by 2-week recovery period	0 (vehicle), 0.75, 1.5 and 3.0	Yes	1704-023	3.0
26-week repeat-dose investigative toxicity study — *Cardiovascular functional evaluation included*	Male and female juvenile cynomolgus monkeys	Weekly dosing for 26 weeks	3.0	No	1704-020	3.0
26-week repeat-dose toxicity study —*Cardiovascular functional evaluation included*	Male and female juvenile cynomolgus monkeys	Weekly dosing for 26 weeks followed by 26-week recovery period	0 (vehicle), 1.2, 2.4 and 4.8	Yes	1704-022	4.8
52-week repeat-dose toxicity study — *Cardiovascular functional evaluation included*	Male and female juvenile cynomolgus monkeys	Weekly dosing for 52 weeks followed by 52-week recovery period	0 (vehicle), 0.40, 1.6 and 4.8	Yes	1704-035	4.8

Type of Study	Species and Strain or Test System	Duration and Administration Schedule	Doses (mg hGH/kg)	GLP Compliance	Study Number	NOAEL (mg hGH/kg)
Genotoxicity Studies						
Bacterial reverse mutation assay (partly cleaved lonapegsomatropin)	Salmonella typhimurium tester strains	Tested with and without S9 activation	300, 600, 1200, 2500 and 5000 µg/plate	Yes	1704-024	NA
Mammalian chromosome aberration assay (partly cleaved lonapegsomatropin)	Human peripheral blood lymphocytes	4 h incubation with and without S9 activation	50, 100, 240, 350 and 500 µg/mL	Yes	1704-025	NA
Reproductive and Development Studies						
A fertility and early embryonic development study	Male and female Sprague Dawley rats	Every 48 h	0 (vehicle), 0.35, 0.70 and 1.4	Yes	9001005	1.4
Embryo-fetal development study	Female Sprague Dawley rats	Weekly on Days 6 and 13 of gestation	0 (vehicle), 0.75, 1.5 and 3.0	Yes	497516	3.0
PK and tolerability study	Female New Zealand White rabbits	Weekly on Days 1 and 8	3.0	No	497390	3.0
Preliminary embryo-fetal development study	Female New Zealand White rabbits	Weekly on Days 6 and 13 of gestation	0 (vehicle), 0.75, 1.5 and 3.0	No	497500	3.0
PK and PD study	Female New Zealand White rabbits	Every 48 h	0.70 and 1.4	No	1704-039	1.4
Embryo-fetal development study	Female New Zealand White rabbits	Every 48 h on Days 7, 9, 11, 13, 15, 17 and 19 of gestation	0 (vehicle), 0.35, 0.70 and 1.4	Yes	9001004	NA[b]
Pre- and postnatal development study (ACP-001)[a]	Female Sprague Dawley rats	Weekly on Days 6, 13 and 20 of gestation and Days 6, 13 and 20 of lactation	0 (vehicle), 0.75, 1.5 and 3.0	Yes	495765	3.0

Type of Study	Species and Strain or Test System	Duration and Administration Schedule	Doses (mg hGH/kg)	GLP Compliance	Study Number	NOAEL (mg hGH/kg)
Local Tolerance						
Assessed as part of studies 1704-033, 1704-037, 1704-023, 1704-022, and 1704-035						
Skin sensitization test (partly cleaved ACP-001)[a]	Local lymph nodes in CBA/J mice	25 µL for 3 days on dorsal side of each ear	25, 2.5 and 0.25 mg/mL	Yes	520487	NA
Ultraviolet-visible-spectrum (b) (4)	In vitro	NA	1.0, 2.0, 5.1 mg/mL	No	TW1104121	NA
Other Pharmacokinetics Studies						
Single dose comparative PK/PD study[c]	Adult male cynomolgus monkeys	Single dose	0.8	No	1704-038	NA

[a] Performed with ACP-001 but considered applicable to lonapegsomatropin
[b] A NOAEL for maternal toxicity or embryo-fetal development could not be determined
[c] Comparison of lonapegsomatropin produced at (b) (4)
CHO = chinese hamster ovary; GLP = good laboratory practice; hERG = human ether-a-go-go-related gene; hGH = human growth hormone; NA = not applicable

Other Toxicity Studies

Type of Study	GLP	Study Number
Toxicological assessment report on possible toxic effects of intermediate compounds in the synthesis of the PEG aromatic linker molecule named 1a and evaluation of the possible breakdown mechanism	No	(b) (4) 2008
Summary and conclusions of DEREK and leadscope model applier assessment of computational toxicity (b) (4) (b) (4)	No	(b) (4) 2011
Computational assessment and evaluation of potential carcinogenicity, genotoxicity and cardiotoxicity of (b) (4)	No	(b) (4) 2011
Computational assessment and evaluation of potential genotoxicity of Linker A impurities using CASE Ultra and DEREK/SARAH	No	(b) (4) -1260, Statement on (b) (4) (b) (4) -1260
Computational assessment and evaluation of potential genotoxicity of Linker A impurities using CASE Ultra and DEREK/SARAH	No	(b) (4) -1260-1374
Literature search on toxicology and pharmacology of (b) (4) (b) (4) (b) (4) and a computational assessment and evaluation of potential genotoxic and non-genotoxic carcinogenicity and developmental and reproductive toxicity using CASE Ultra and DEREK	No	(b) (4) 1272-1352
Computational assessment and evaluation of the potential genotoxicity of 5 (b) (4) impurities, (b) (4) using CASE Ultra and DEREK/SARAH	No	(b) (4) 1297
Computational Assessment and Evaluation of Potential Bacterial Mutagenicity (b) (4), (b) (4)	No	(b) (4) -1350-1383

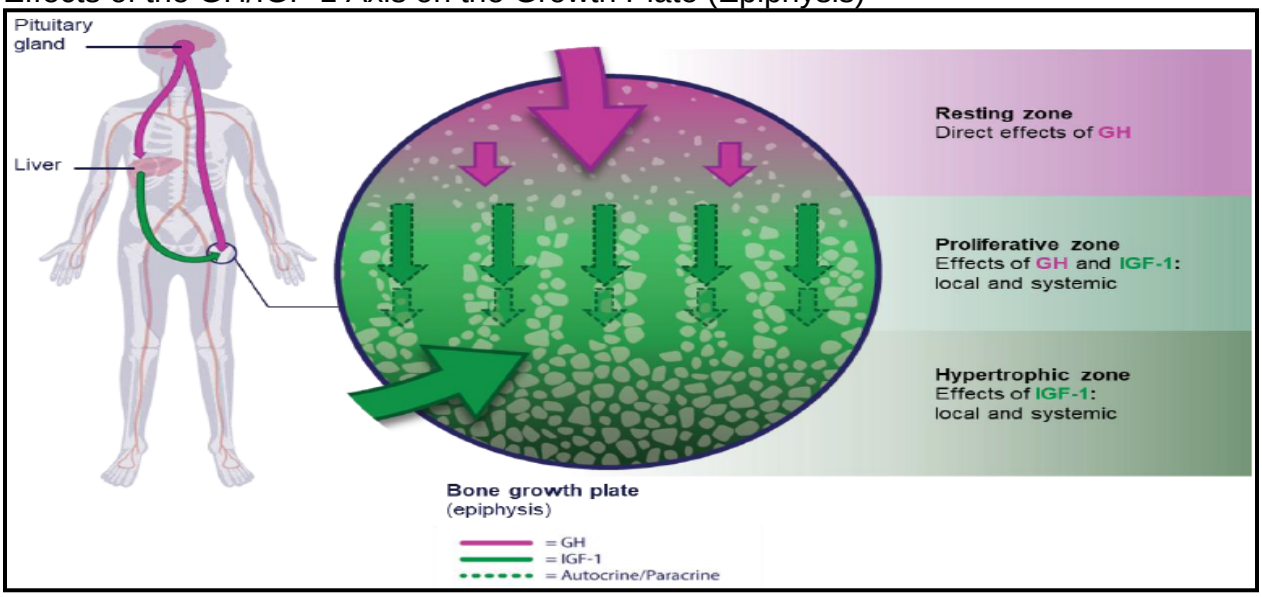
4 Pharmacology

4.1 Primary Pharmacology

Growth hormone deficiency (GHD) is typically caused by defects arising in the pituitary gland or in the hypothalamus.

Human growth hormone (hGH) is normally produced by the somatotroph cells of the anterior pituitary gland. The secretion of GH from the pituitary gland is stimulated by growth hormone releasing hormone (GHRH) and inhibited by somatostatin, both of which are produced by the hypothalamus. GH elicits a broad spectrum of pharmacological effects in the human body and is essential in promoting bone growth. GH exerts numerous direct effects in peripheral tissues and induces hepatic insulin-like growth factor-1 (IGF-1) as well as local IGF-1 production in the growth plate.

Effects of the GH/IGF-1 Axis on the Growth Plate (Epiphysis)



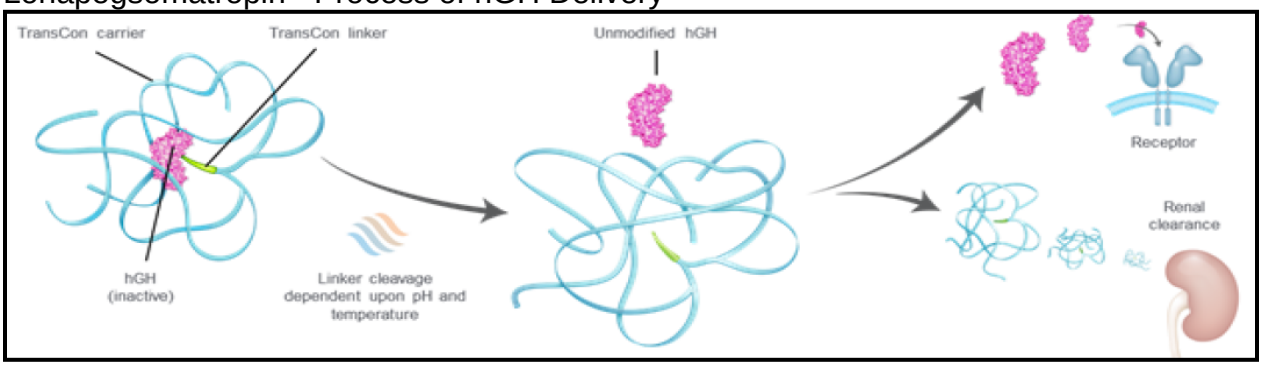
Lonapegsomatropin is a long-acting hGH pro-drug intended to release somatropin with the same mode of action (including tissue penetration and receptor binding affinity) and distribution as daily hGH, but via a once-weekly subcutaneous injection.

(In Vitro)

In vitro pharmacology studies (analytical comparability, cell-based proliferation/potency assays and binding and disassociation kinetics studies) demonstrated that the lonapegsomatropin pro-drug (hGH bound to carrier) exhibits reduced binding to the hGH receptor and minimal in vitro activity compared to unconjugated hGH.

The hGH released from lonapegsomatropin (following hydrolysis) is fully active (unmodified recombinant hGH) and elicits biopotency comparable to somatropin.

Lonapegsomatropin - Process of hGH Delivery

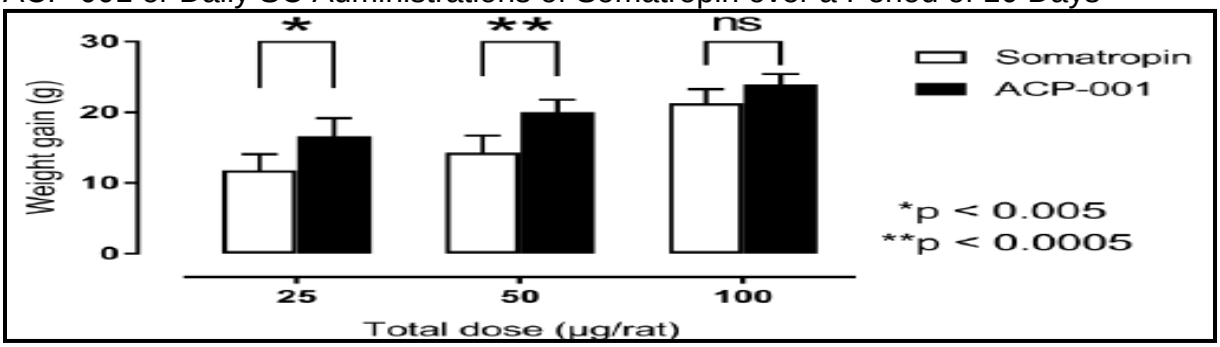


In vitro studies employing ACP-001 (predecessor of lonapegsomatropin) demonstrated that the pharmacology of lonapegsomatropin and ACP-001 were comparable with regards to the mass and integrity of released hGH, in vitro dissociation (k_d) from the human growth hormone receptor (hGHR) and potency of released hGH. In vitro binding (k_a) to hGHR was slightly lower for ACP-001 and was considered a result of the larger mPEG chains in ACP-001. Hence, early nonclinical studies conducted with the predecessor molecule, ACP-001, were deemed relevant for safety evaluation of lonapegsomatropin.

(In Vivo)

In vivo pharmacology studies were conducted in hypophysectomized male SD rats and normal Cynomolgus monkeys to evaluate the pharmacodynamic activity of lonapegsomatropin relative to daily somatropin. Lonapegsomatropin (once weekly dosing) resulted in sustained exposure to hGH that elicited a larger increase in systemic IGF-1 levels in Cynomolgus monkeys and greater increases in body weight gain in male hypophysectomized rats, compared to once-daily administration of somatropin.

Total Body Weight Gain of Hypophysectomized Rats After 3 SC Administrations of ACP-001 or Daily SC Administrations of Somatropin over a Period of 10 Days



This data is consistent with the results from repeat-dose toxicity and DART studies where lonapegsomatropin increased IGF-1 levels (above endogenous baseline), increased body/organ weights and/or altered metabolic parameters (indicative of lipolysis) in Sprague Dawley rats (females), New Zealand White rabbits (females) and/or Cynomolgus monkeys (adults and juveniles).

Body weight decreased in male rats during the 4-Week toxicity study and correlated with a reduction in food intake. Body weight gains in males were comparable to controls during the 26-Week pivotal toxicity study where HD males generally consumed less food than control males. The mechanism driving weight loss/inappetence in male rats is unclear; however, it was likely related to the decrease in adipose tissue as a result of exposure to hGH in male SD rats.

4.2 Secondary Pharmacology

No secondary pharmacodynamic effects were observed during repeat-dose toxicity studies in rats and monkeys employing supraphysiologic levels of lonapegsomatropin and no dedicated secondary pharmacology studies were conducted.

4.3 Safety Pharmacology

Safety pharmacology studies included an in vitro evaluation of ACP-001 (predecessor of lonapegsomatropin) in a hERG assay and in vivo studies evaluating the potential effects of lonapegsomatropin on the central nervous system (CNS) and respiratory system in Sprague Dawley rats and the cardiovascular (CV) system in Cynomolgus monkeys. No treatment-related effects were observed in the CNS, Respiratory or CV systems.

Cardiovascular Evaluation

(In Vitro - hERG Assay - GLP)

Exposure of hERG channels to hydrolyzed ACP-001 (lonapegsomatropin predecessor), hGH, mPEG (b) (4) and (b) (4), failed to elicit inhibitory effects on hERG conductance. The levels of ACP-001 drug-cleavage products (hGH, mPEG (b) (4) and (b) (4)) at the no observed effect level (NOEL) were significantly higher than those associated with the maximal concentrations (C_{max}) observed in human plasma following the administration of lonapegsomatropin at 0.24 mg hGH/kg/week.

Comparison of hERG Assay to Human Concentrations of hGH, mPEG and (b) (4)

	hGH (ng/mL)	mPEG (ng/mL)	(b) (4) (ng/mL)
hERG Assay NOEL Concentration	72000	289000	235
Clinical C_{max} at 0.24 mg hGH/kg/QW (Steady State)	18.5	13100	< LLOQ (25 pg/mL)
Multiple of Exposure	**3891**	**22**	**> 9400**

(In Vivo - GLP)

Cardiovascular effects were evaluated as part of the repeat-dose toxicity studies in adult and juvenile monkeys at multiple time points to ensure adequate coverage of the t_{max} associated with the parent (pro-drug) and the drug-cleavage products (hGH, mPEG, and (b) (4)). No effect was observed on the electrocardiography (ECG) parameters (qualitative or quantitative) assessed following SC administration of lonapegsomatropin at doses ≤ 4.8 mg hGH/kg/week for ≤ 52 weeks.

Respiratory and Neurobehavioral Evaluations

(In Vivo - GLP)

No adverse effects were observed in the respiratory and central nervous system during the initial 24 hr period (acute effects) and on Day 7 following a single SC dose ≤ 3 mg hGH/kg/dose (maximum dose level assessed) in the dedicated safety pharmacology studies (GLP) conducted in male rats.

These findings are consistent with the absence of adverse clinical, CNS or respiratory findings in the repeat-dose toxicity studies of lonapegsomatropin conducted in SD rats or cynomolgus monkeys at doses ≤ 4.8 mg hGH/kg/week (maximum dose assessed) that included an assessment of juvenile monkeys for up to 52 weeks (QW).

Renal Evaluation

(In Vivo)

Clinical chemistry and urinalysis assessments were included in the repeat-dose toxicity studies in rats and monkeys to evaluate the potential effects of lonapegsomatropin on the renal/urinary system. These parameters were assessed at pre-dose, 24-48 hrs post-dose (mid-study) and at necropsy or during Week 1, 26, 39, and 52 in the 52-week study in juvenile cynomolgus monkeys. No test-article related changes in urinalysis or associated clinical chemistry parameters were observed in either sex at any dose level in the 4- or 27-Week rat studies or in the 4-, 26-, or 52-Week monkey studies.

Comparison of C_{max} of Lonapegsomatropin, hGH, mPEG and (b) (4) in Safety Pharm Studies in Rats and Monkeys with Clinical Exposure Levels

	Lonapegsomatropin (ng hGH/mL)	**hGH (ng/mL)**	**mPEG (ng/mL)**	**(b) (4) (pg/mL)**
Safety Pharm C_{max} - 3 mg hGH/kg Male Rat	3590	20.1	15000	< LLOQ (75 pg/mL)
Repeat-Dose Tox C_{max}-4.8 mg hGH/kg QW - 52W Monkey	37300	320	425000	74.9
Clinical C_{max} at 0.24 mg hGH/kg/QW (Steady State)	1230	18.5	13100	< LLOQ (25 pg/mL)
Exposure Multiples				
(Rat/Human)	**2.9**	**1.1**	**1.1**	**N/D**
(Monkey/Human)	**30**	**17**	**32**	**> 3**

Abbreviations: Lower Limit of Quantification (LLOQ), Not Determined (N/D)

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

No formal nonclinical absorption, distribution, metabolism or excretion studies were conducted for lonapegsomatropin. The following analytes were assessed in the systemic circulation in SD rats, Cynomolgus monkeys and New Zealand White rabbits:

- Lonapegsomatropin (pro-drug)
- hGH (active component of lonapegsomatropin)
- mPEG (carrier molecule). Total mPEG was quantified (i.e., both the pro-drug and the released mPEG).
- (b) (4) (TransCon linker leaving group released during autocleavage)
- mPEG-linker ((b) (4) TransCon linker part attached to mPEG)
- IGF-1 (Biomarker for Growth Hormone Receptor (GHR) activation)
- Anti-hGH (ADAs against the active component of lonapegsomatropin)
- Anti-PEG (ADAs against the carrier molecule)
- Neutralizing anti-hGH (Neutralizing ADAs against the active component of lonapegsomatropin).

Absorption

Formal bioavailability studies in rats and monkeys were not conducted.

The mean half-life of lonapegsomatropin (in hGH equivalents) and baseline corrected hGH ranged from 22 hrs to 67 hrs and 31 hrs to 66 hrs, respectively, in juvenile cynomolgus monkeys administered lonapegsomatropin (QW) by SC administration at dose levels of 0, 0.4, 1.6 and 4.8 mg hGH/kg/week for 52 weeks. The half-life of lonapegsomatropin/hGH was not determined in SD rats.

The mean half-life (hGH) in GHD pediatric patients was 20.6 hrs (steady state) following once weekly treatment of lonapegsomatropin (0.24 mg hGH/kg/week) compared to a half-life of 2.5 hrs (steady state) for daily unmodified hGH (0.034 mg hGH/kg/day).

(mPEG)

The terminal half-life for mPEG ranged from 485 to 950 hrs (20 to 40 days) in SD rats administered lonapegsomatropin (QW) by SC administration at doses of 1.2, 2.4 and 4.8 mg hGH/kg/week for 27 weeks. The mean half-life ($t_{1/2}$) for mPEG ranged from 370 to 1310 hrs (15 to 54 days) in juvenile Cynomolgus monkeys administered lonapegsomatropin (QW) by SC administration at doses of 0.4, 1.6 and 4.8 mg hGH/kg/week for 52 weeks ($t_{1/2}$, 465 hrs to 723 hrs, 26 Week Juv. Monkey, ≤ 4.8 mg).

Metabolism/Excretion

Hydrolysis of lonapegsomatropin results in the release of hGH and two additional cleavage products: mPEG (b) (4) and (b) (4)

Lonapegsomatropin Hydrolysis and Breakdown Components

(b) (4)

Recombinant hGH (released from lonapegsomatropin pro-drug) will likely be metabolized in the same manner as endogenous GH, where GH is catabolized in the liver and kidney to its constitutive amino acids.

Given the chemical structure (b) (4) metabolism by hepatic enzymes (cytochrome P450 or flavin containing monooxygenases) that lead to the oxidation and/or dealkylation (b) (4) is considered likely. Metabolism (b) (4) is thus expected to result in metabolites of a hydrophilic nature that would likely be excreted renally. (b) (4) was rapidly cleared and did not accumulate in Cynomolgus monkeys following repeat-dosing for ≤ 52 weeks (C_{max}, 74.9 pg/mL) at 4.8 mg hGH/kg/week (HD) and was generally not detectable (BLQ) in humans, rats and rabbits following repeat-dose administration.

(b) (4)

While mPEG is expected to be primarily excreted in the urine by renal filtration, the PEG backbone is prone to oxidative degradation via cellular phagocytosis. This mechanism is expected to play a role in the elimination of mPEG (b) (4) and will likely facilitate clearance from tissues.

Distribution

Dedicated distribution studies of lonapegsomatropin were not conducted. Pivotal rat and monkey chronic repeat-dose toxicity studies included immunohistochemical (IHC) assessments for the presence of hGH (monkey only) and mPEG in the final injection site and selected areas of the brain and are described in the general toxicology section.

(Choroid Plexus – mPEG)

Steady state levels of mPEG in the systemic circulation of monkeys were reached following 13 weeks of repeat-dosing. Systemic mPEG levels were observed to be approaching steady state levels at or before 6 months of treatment during the Phase 3 trial in children with GHD (CT-301).

Population pharmacokinetic modeling of mPEG across species and published mPEG data in serum and the choroid plexus of rats following administration of a 40 kDa PEG molecule was employed to predict the mPEG exposure in choroid plexus of pediatric subjects following dosing with lonapegsomatropin. The predicted time to reach steady state in the systemic circulation of GHD children is 3 months and the time to reach steady state in the choroid plexus is estimated to be 16 months, consistent with the slow distribution of mPEG between these 2 compartments.

The predicted median steady state level of mPEG in the choroid plexus of GHD children administered lonapegsomatropin at 0.24 mg hGH/kg/week is 2-fold lower than the predicted steady state levels in the choroid plexus of monkeys at the no observed effect level (NOEL, 0.4 mg hGH/kg/week, LD) for microscopic findings (H&E) noted in the brains (vacuolation of epithelial cells and/or macrophages within the choroid plexus) of both sexes at doses ≥ 1.6 mg hGH/kg/week (MD/HD) for 52 weeks.

The model predicts that 90% of mPEG will be cleared from the systemic circulation in GHD children within approximately 3 months from end of treatment (based on the predicted systemic half-life of 26 days) and 90% of mPEG will be cleared from choroid plexus within 16 months (based on the predicted half-life of approximately 5 months).

(Cerebral Spinal Fluid - mPEG)

No mPEG was detected in the cerebral spinal fluid (CSF) of SD rats or Cynomolgus monkeys where levels observed in the systemic circulation at the HD were 41-, 106- and 634-fold higher than the LLOQ in the CSF of male rats, female rats and monkeys, respectively.

Drug-Drug Interactions

No formal drug interaction studies were conducted with lonapegsomatropin, mPEG or (b) (4) As mPEG is considered to be biologically inert (post-methoxylation) and (b) (4) levels were below the LLOQ at clinical steady state (0.24 mg hGH/kg/week dose) the drug interaction potential of these drug cleavage products were not assessed but would be minimal. Thus, drug interactions with lonapegsomatropin are anticipated to be the same as for recombinant hGH.

6 General Toxicology

Pivotal repeat-dose toxicity studies included 4- and 27-Week GLP toxicity studies in Sprague Dawley rats, and 4-(adult), 26- and 52-Week (juvenile) GLP toxicity studies in Cynomolgus monkeys. Weekly administration of lonapegsomatropin was well tolerated and no adverse findings were observed in repeat-dose toxicity studies conducted in SD rats and Cynomolgus monkeys. Hence, the no observed adverse effect level (NOAEL) was considered to be 4.8 mg hGH/kg/week for the pivotal toxicity studies conducted in SD rats and Cynomolgus monkeys (the highest dose assessed in both species).

To evaluate the safety and distribution of mPEG, histopathological assessments included H&E staining of the CNS (cerebrum, midbrain, cerebellum [including choroid plexus], medulla/pons and spinal cord) and peripheral (sciatic nerve) nervous system. The histopathological assessment also incorporated immunohistochemical (IHC) evaluations for hGH (monkey) and mPEG (rat and monkey) in multiple brain regions known to express high levels of growth hormone receptor (GHR) or to be highly vascularized, including several circumventricular organs (CVOs). Detailed clinical observations focusing on the CNS function were also evaluated.

As the findings in the repeat-dose toxicity studies were generally comparable across the studies conducted in SD rats or cynomolgus monkeys, the following discussion is restricted to the chronic repeat-dose toxicity studies (27-Week SD Rat, 1704-037 and 52-Week juvenile cynomolgus monkey, 1704-035).

6.2 Repeat-Dose Toxicity

(Rat – 27-Week Pivotal Repeat-Dose Toxicity - 1704-037)

The 27-Week repeat-dose rat toxicity study (Pivotal, GLP) evaluated lonapegsomatropin doses of 1.2, 2.4, and 4.8 mg hGH/kg/week in 15 rats/sex/group, with an additional 6 rats/sex/group being assessed in a 27-week recovery phase. Sprague Dawley rats were 6-8 weeks at study initiation and reached adulthood during the 27-week dosing period.

SD Rats – PK/PD

Systemic exposure to lonapegsomatropin, hGH, mPEG, and IGF-1, following acute dosing (Day 1), was sex-dependent with higher systemic exposure in males compared to females, but following repeat-dosing, exposures tended to decline more rapidly in males and resulted in higher exposure levels in females from Week 7 forward. Rats with

systemic exposure to lonapegsomatropin, in general, demonstrated systemic exposure to hGH, with associated IGF-1 concentrations above baseline (Indicative of PD activity).

Anti-drug antibodies (ADAs) specific for human growth hormone (anti-hGH) were detected in the majority of SD rats following repeat-dose administration of lonapegsomatropin and were predominantly neutralizing (correlating with a decline in lonapegsomatropin/hGH exposure and loss of a PD response, IGF-1).

Week 26 - Systemic Exposure (Mean) after SC Administration of Lonapegsomatropin to SD Rats at 4.8 mg hGH/kg/Week (1704-037)

Analyte	Sex	C_{24h} (min-max)	C_{max} (µg/mL)	$AUC_{0\text{-}168h}$ (h*µg/mL)
Lonapegsomatropin (ng hGH/mL)	M	251 (103 – 465)	NA	NA
	F	686 (7.30 – 1,770)	NA	NA
hGH baseline corrected (ng/mL)	M	13.8 (8.35 – 22.9)	NA	NA
	F	25.7 (<2-00 – 95.4)	NA	NA
mPEG (µg/mL)	M	33.2 (12.6 – 66.9)	48.5	6,070
	F	47.1 (26.4 – 64.9)	61.0	8,840

SD Rats – Mortality/Clinical Observations

No lonapegsomatropin-related mortalities were observed, and no adverse findings were noted in the clinical observations (including CNS evaluations for tremors, convulsions, reactivity to handling and unusual behavior), dermal scoring, ophthalmology, ECGs, hematology, coagulation, urinalysis and macroscopic assessments conducted during the administration (27-Weeks) and recovery periods (27-Weeks) in SD rats.

SD Rats – mPEG – Systemic Exposure

Systemic exposure to mPEG increased in a dose-proportional manner and was minimally affected by the presence of anti-hGH antibodies. There was no increase in mPEG exposure from Week 13 to 26 indicating that steady state had likely been reached at ≤ 13 weeks of lonapegsomatropin dosing (QW). Following the last dose, the terminal half-life for mPEG ranged from 20 to 40 days, suggesting that it would take 14 to 28 weeks (3.5 to 7 months) to achieve 5 half-lives. mPEG was still quantifiable in the majority of HD rats after 17 weeks (4.25 months) of recovery (last sampling point).

SD Rat - Cerebral Spinal Fluid - mPEG

A quantitative analysis revealed that mPEG was not detectable in the CSF samples collected at necropsy from main and recovery SD rats (LLOQ of 500 ng/mL).

SD Rat – mPEG Immunohistochemical (IHC) Analysis

The distribution of mPEG was assessed by immunohistochemical (IHC) staining of the injection site and brain tissues from the 27-Week repeat-dose toxicity study. IHC staining was performed on the brains (≤ 4 tissue sites/rat) from all control and

lonapegsomatropin-dosed rats at the end of dosing and following recovery. Vehicle-dosed rats were negative for mPEG staining consistent with the lack of PK/PD.

SD Rat - Injection Site – mPEG (IHC)

mPEG staining (IHC) associated with the final injection site manifested in multiple tissue types/structures in lonapegsomatropin-dosed rats including the intravascular, endothelium, vascular wall and interstitium in the dermis and/or subcutis, macrophages, hair follicle epithelium and/or sebaceous gland epithelium. In general, the mPEG staining at the injection site was described as finely granular and consistent with physiologic (vascular and interstitial) distribution and macrophage/epithelial cell uptake and revealed mPEG positive vacuoles in tissue resident macrophages. mPEG stained granules/vacuoles observed in macrophages derived from the final injection site were not associated with a distortion of the cytoplasmic or nuclear compartments, cellular degeneration or tissue necrosis in lonapegsomatropin-dosed rats (not adverse).

SD Rats - Brain – mPEG (IHC)

mPEG staining (IHC) was observed in the choroid plexus and brain tissues associated with blood brain barrier function in lonapegsomatropin-dosed rats. The mPEG staining observed in the brain was described as fine-bore granules and vacuoles in the cytoplasm of choroid plexus epithelial cells, as well as fine-bore granules in the cytoplasm of ependymal cells lining the third ventricle, in specialized glial cells in the median eminence/infundibulum surrounding the base of the third ventricle or in the adjacent hypothalamus in main study and recovery SD rats.

mPEG granules in the cytoplasm of choroid plexus endothelial cells manifested only in MD/HD recovery rats and was likely driven by the persistent (albeit declining) systemic exposure to mPEG ($t_{1/2}$, 20 to 40 days) during the 27-week recovery period.

mPEG (IHC) Staining in Brain Tissues in Main Study and Recovery Period SD Rats after 27 Weeks of Lonapegsomatropin Administration (1704-037)

	Dose Level	Main				Recovery			
Brain Tissue	mg hGH/kg/week	Males	Females	Intensity	Frequency	Males	Females	Intensity	Frequency
Choroid Plexus, Epithelium	1.2	5/15	12/15	Minimal to mild	Very rare to rare	6/6	6/6	Minimal to mild	Very rare to rare to occasional
	2.4	14/15	15/15	Minimal to mild	Very rare to rare to occasional	6/6	5/5	Minimal to mild	Very rare to rare to occasional
	4.8	14/14	14/14	Minimal to mild	Very rare to occasional	5/5	6/6	Minimal to mild	Very rare to occasional
Choroid Plexus, Endothelium	1.2	0/15	0/15	-	-	0/6	0/6	-	-
	2.4	0/15	0/15	-	-	1/6	1/5	Minimal to mild	Very rare
	4.8	0/14	0/14	-	-	3/5	4/6	Minimal to mild	Very rare
Specialized Glial Cells	1.2	1/15	1/15	Minimal to mild	Very rare	2/6	3/6	Minimal to mild	Very rare
	2.4	2/15	2/15	Minimal to mild	Very rare	2/6	4/5	Minimal to mild	Very rare
	4.8	1/14	9/14	Minimal to mild	Very rare to rare	3/5	4/6	Minimal to mild	Very rare to rare

Ependymal Cells	1.2	0/15	0/15	-	-	1/6	0/6	Minimal to mild	Very rare
	2.4	0/15	3/15	Minimal to mild	Very rare	0/6	0/5	-	-
	4.8	3/14	2/14	Minimal to mild	Very rare	0/5	2/6	Minimal to mild	Very rare

Table presents number of positive animals/number of animals examined
Intensity scores: Negative = no staining, <1+ = very minimal/trace, 1+ = minimal, 2+ = mild, 3+ = moderate, 4+ = marked (intense)
Frequency scores: Frequency modifiers reflected the approximate percentage staining of that cell type or tissue element at that location, as follows: no staining, very rare (<1% of scored element, eg, cell type, extracellular location, or other tissue element), rare (1-5% of scored element), rare to occasional (>5-25% of scored element), occasional (>25-50% of scored element), occasional to frequent (>50-75% of scored element), frequent (>75-100% of scored element)

The incidence, intensity and/or frequency of mPEG staining was generally comparable at the MD/HD (end of dosing vs recovery) but tended to increase at the LD in choroid plexus epithelial cells and specialized glial cells in recovery rats, indicating that mPEG continued to accumilate in these cell types in the absence of dosing at the LD.

mPEG (IHC) Stained Vacuoles in the Choroid Plexus Epithelial Cells in Main Study and Recovery Period Rats after 27 Weeks of Lonapegsomatropin Administration (1704-037)

	Dose level	**Main**		**Recovery**	
	(mg hGH/kg/week)	**Males**	**Females**	**Males**	**Females**
Choroid Plexus, Epithelium	1.2	1/15	2/15	6/6	2/6
	2.4	7/15	8/15	6/6	5/5
	4.8	9/14	13/14	5/5	6/6

mPEG (IHC) stained granules/vacuoles observed in the choroid plexus and other brain tissue elements related to blood brain barrier function were not associated with a distortion of the cytoplasmic or nuclear compartments, cellular degeneration or tissue necrosis in lonapegsomatropin-dosed SD rats (not adverse).

Note: Standard light microscopy of H&E stained slides was insufficient to observe vacuolation in any tissue, including the choroid plexus, in SD rats.

The Reviewer considers the HD (4.8 mg hGH/kg/QW) the most clinically relevant NOAEL as the subcutaneous administration of lonapegsomatropin to SD rats (1.2, 2.4, and 4.8 mg hGH/kg/QW) for 27 weeks followed by a 27-week recovery period resulted in no adverse findings.

(Juvenile Cynomolgus Monkey – 52-Week Pivotal Repeat-Dose Toxicity - 1704-035)

The 52-Week repeat-dose juvenile monkey toxicity study (Pivotal, GLP) evaluated lonapegsomatropin at doses of 0.4, 1.6, and 4.8 mg hGH/kg/week in 4 monkeys /sex/group. Additionally, 2 monkeys/sex/group were assessed during a 52-week recovery period. Juvenile cynomolgus monkeys were 13-17 months at study initiation.

Cynomolgus Monkey – PK/PD

Systemic exposure to lonapegsomatropin, hGH and mPEG was observed in monkeys administered lonapegsomatropin (sex-independent) and elicited a dose-related IGF-1 response (PD activity). (b) (4) was rapidly eliminated from systemic circulation and did not accumulate during the 52-week dosing period in cynomolgus monkeys.

Anti-drug antibodies (ADAs) specific for human growth hormone (anti-hGH) or mPEG (anti-PEG) were not detected in cynomolgus monkeys dosed with lonapegsomatropin for ≤ 52-Weeks (QW).

Mean Systemic Exposure at Week 52 after SC Administration of Lonapegsomatropin to Juvenile Cynomolgus Monkeys at 4.8 mg hGH/kg/Week (1704-035)

Analyte	Sex	C_{max} (µg/mL)	$AUC_{0\text{-}168h}$ (h*µg/mL)
Lonapegsomatropin (µg hGH/mL)	M+F	37.3	3,850
hGH baseline corrected (µg/mL)[a]	M+F	0.320	26.1
mPEG (µg/mL)	M+F	425	58,900

[a] Week 1 data presented for hGH, due to assay interference at Week 52

Cynomolgus Monkey – Mortality/Clinical Observations

No lonapegsomatropin-related mortalities were observed, and no adverse findings were noted in clinical observations (including CNS evaluations for tremors, convulsions, reactivity to handling and unusual behavior), dermal scoring, ophthalmology, ECGs, hematology, coagulation and urinalysis during the administration (52-Weeks) and recovery periods (52-Weeks) in cynomolgus monkeys.

Body weights tended to increase in lonapegsomatropin-dosed monkeys and were associated with minimal changes in organ weights (absolute and relative) that were consistent with the pharmacological effect of hGH. These findings did not elicit any notable microscopic changes in the affected organs (liver, thyroid, parathyroid glands, adrenal glands and/or kidneys) and were not considered adverse.

Cynomolgus Monkey – Microscopic Findings (H&E Staining) - Mammary Gland

Microscopic findings in the mammary gland were observed in lonapegsomatropin-dosed monkeys and included mammary duct/gland dilation (galactocele) with or without luminal exudate, lobular hyperplasia, mononuclear cell infiltration and increased secretory vacuolation of the glandular epithelial cells in HD males and MD/HD females. These conditions tended to resolve during recovery and were not considered adverse but related to the known effects of excessive growth hormone activity on mammary tissue.

Cynomolgus Monkey – Microscopic Findings (H&E Staining) - Brain

Vacuolation in choroid plexus epithelial cells and/or macrophages was observed (H&E staining, light microscopy) in lonapegsomatropin-dosed monkeys (MD/HD, both sexes) and tended to increase in incidence and/or severity with ascending dose.

The vacuolation noted in choroid plexus epithelial cells persisted in MD/HD recovery monkeys (both sexes) while macrophage vacuolation tended to resolve. The vacuolation observed in epithelial cells and macrophages of the choroid plexus did not elicit structural changes in the brain tissue architecture and are not expected to have resulted in functional impairment in cynomolgus monkeys.

Choroid Plexus Microscopic Observations (H&E) in Main Study and Recovery Period Monkeys after 52 Weeks of Lonapegsomatropin Administration (1704-035)

Dose level (mg hGH/kg/week)	**0**		**0.40**		**1.6**		**4.8**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Main Animals, Number Examined	**4**	**3**	**3**	**4**	**4**	**4**	**4**	**4**
Brain								
Epithelium, Vacuolated	0	0	0	0	1	2	4	3
-Minimal	0	0	0	0	1	2	3	3
-Mild	0	0	0	0	0	0	1	0
Macrophages, Vacuolated								
-Minimal	1	0	0	0	0	4	4	3
Recovery Animals, Number Examined	2	2	2	2	2	2	2	2
Epithelium, Vacuolated	0	0	0	0	2	2	2	2
-Minimal	0	0	0	0	2	2	1	0
-Mild	0	0	0	0	0	0	1	2
Macrophages, Vacuolated								
-Minimal	0	0	0	0	0	0	1	0

M-Male; F-Female

Cynomolgus Monkeys - mPEG – Systemic Exposure

The mPEG exposure data from the 26-Week and 52-Week studies suggests that steady state is essentially reached at ≤ 13 weeks in cynomolgus monkeys (A 2- to 4-fold accumulation of systemic mPEG was observed at steady state in monkeys).

mPEG was eliminated from systemic circulation in monkeys with a mean half-life that ranged from 370 hrs (15 days) to 1310 hrs (55 days). A bi-phasic elimination was observed at the HD, the slower phase was considered to be consistent with the elimination from tissues with slow turnover rate and was likely detectable only at the HD due to the limitations of the assay (< LLOQ). At the end of the 52-week recovery period, all but 2 recovery monkeys had mPEG levels below the LLOQ (500 ng/mL).

Cynomolgus Monkey - Cerebral Spinal Fluid - mPEG

A quantitative analysis revealed that mPEG was not detected in CSF samples collected at necropsy from main study and recovery cynomolgus monkeys (LLOQ of 500 ng/mL).

Cynomolgus Monkey – mPEG Immunohistochemical (IHC) Analysis

Brain tissue sections derived from cynomolgus monkeys (consecutive with those prepared and stained with H&E) were selected based on prior structure identification and stained for mPEG (IHC). mPEG staining of brain tissues from vehicle control monkeys was negative (consistent with the absence of PK/PD).
Cynomolgus Monkey - Brain – mPEG (IHC)

mPEG staining was observed in brain tissue elements in the lining of the blood brain barrier and CSF barrier in lonapegsomatropin-dosed monkeys.

The frequency of mPEG staining of cytoplasmic granules and vacuoles in choroid plexus epithelial cells was notably reduced and fully resolved in the third ventricle following the 52-week recovery period. Full reversibility of mPEG staining was observed in the intravascular space, while partial reversibility was noted in the specialized glial cells, interstitial macrophages, ependymal cells and endothelium.

mPEG (IHC) Staining in Brain Tissue Elements in Main Study and Recovery Period Monkeys after 52 Weeks of Lonapegsomatropin Administration (1704-035)

	Dose Level	Main				Recovery			
Brain Tissue	mg hGH/kg/week	Males	Females	Intensity	Frequency	Males	Females	Intensity	Frequency
Choroid Plexus, Epithelium	0.40	3/3	4/4	Minimal to mild	Very rare to occasional	2/2	2/2	Minimal to moderate	Very rare to occasional
	1.6	4/4	4/4	Minimal to moderate	Occasional to frequent	2/2	2/2	Minimal to moderate	Rare to frequent
	4.8	4/4	4/4	Minimal to moderate	Frequent	2/2	2/2	Minimal to moderate	Rare to frequent
Choroid Plexus, Macrophages	0.40	1/3	0/4	Minimal to mild	Very rare	0/2	0/2	-	-
	1.6	0/4	1/4	Minimal to mild	Very rare	0/2	0/2	-	-
	4.8	4/4	4/4	Minimal to moderate	Rare to occasional	1/2	0/2	Minimal to mild	Rare
Specialized Glial Cells	0.40	1/3	1/4	Minimal	Very rare	0/2	0/2	-	-
	1.6	2/4	2/4	Minimal	Very rare to rare	0/2	0/2	-	-
	4.8	4/4	3/4	Minimal to mild	Very rare to occasional	0/2	1/2	Minimal to mild	Rare
Ependymal Cells	0.40	0/3	0/4	-	-	0/2	0/2	-	-
	1.6	0/4	0/4	-	-	1/2	0/2	Minimal to moderate	Rare
	4.8	4/4	0/4	Minimal to moderate	Very rare to occasional	0/2	1/2	Minimal to moderate	Very rare to frequent

Endothelium	0.40	0/3	0/4	-	-	0/2	0/2	-	-
	1.6	0/4	0/4	-	-	0/2	0/2	-	-
	4.8	4/4	4/4	Minimal to mild	Very rare to rare	2/2	0/2	Minimal to mild	Very rare to rare
Intravascular	0.40	0/3	0/4	-	-	0/2	0/2	-	-
	1.6	1/4	0/4	Minimal to mild	Very rare	0/2	0/2	-	-
	4.8	4/4	4/4	Minimal to mild	Very rare to occasional	0/2	0/2	-	-

Table presents number of positive animals/number of animals examined
Intensity scores: Negative = no staining, <1+ = trace/very minimal, 1+ = minimal, 2+ = mild (slight), 3+ = moderate, 4+ = marked (intense)
Frequency scores: Frequency modifiers reflected the approximate percentage staining of that cell type or tissue element at that location, as follows: no staining, very rare (<1% of scored element, eg, cell type, extracellular location, or other tissue element), rare (1-5% of scored element), rare to occasional (>5-25% of scored element), occasional (>25-50% of scored element), occasional to frequent (>50-75% of scored element), frequent (>75-100% of scored element)

A detailed description of mPEG staining (IHC) in the monkey brain is included below:

- mPEG stained cytoplasmic granules and/or vacuoles were present in choroid plexus epithelium and/or interstitial macrophages at all doses and mPEG stained macrophages were noted in the leptomeninges in 1 HD monkey.

- Specialized glial cells stained for mPEG (at all doses) at the level of the median eminence infundibulum and/or in the neuropil generally just beneath the ependyma lining the lateral or third ventricle.

- mPEG stained granules were present in the cytoplasm of ependymal cells of the base of the third and/or fourth ventricle in HD males. mPEG staining (minimal, rare) located as fine-bore granules in the perikaryon of 5 neurons adjacent to the third ventricle were observed in a single HD male.

- mPEG staining of the blood vessel endothelium in the choroid plexus, neuropil (cerebrum and/or cerebellum) and/or leptomeninges was predominantly diffuse and cytoplasmic, although, fine-bore granules and/or vacuoles also were noted.

- Blood vessel mPEG staining (intravascular/finely granular) was observed in the neuropil (1 MD male) and in the neuropil, choroid plexus, outer molecular layer, leptomeninges and/or in proximity to the median eminence/infundibulum (HD).

mPEG staining was generally observed as finely granular although in a subset of cell types, vacuoles were notably present (See Below). The mPEG stained (IHC) vacuoles were predominantly small and were likely undetectable by routine H&E staining.

mPEG (IHC) Stained Vacuoles in Brain Tissue Elements in Main Study and Recovery Period Monkeys after 52 Weeks of Lonapegsomatropin Administration (1704-035)

	Dose Level (mg hGH/kg/week)	Number of Animals	
		Main	Recovery
Choroid plexus, Epithelial Cells	0.40	7/7	4/4
	1.6	8/8	4/4
	4.8	8/8	4/4
Choroid plexus, Macrophages	0.40	0/7	0/4
	1.6	1/8	0/4
	4.8	8/8	1/4
Specialized Glial Cells	0.40	1/7	0/4
	1.6	2/8	0/4
	4.8	6/8	0/4
Endothelium, Neuropil	0.40	0/7	0/4
	1.6	0/8	0/4
	4.8	2/8	0/4

Table presents number of positive animals/number of animals examined

In the absence of distortion of the cytoplasmic or nuclear compartments, degeneration or necrosis of cells containing mPEG stained granules or vacuoles, or the surrounding cells and/or tissues, these observations were not considered adverse. Apart from areas in the cytoplasm with vesicles containing mPEG, cells derived from lonapegsomatropin-dosed or control monkeys were morphologically comparable. As noted above, mPEG staining and/or vacuolation was not associated with any adverse clinical signs including CNS evaluations for tremors, convulsions, reactivity to handling and unusual behavior.
Cynomolgus Monkey – Brain - hGH (IHC)

To differentiate between mPEG staining associated with mPEG released from lonapegsomatropin and mPEG conjugated to hGH, hGH (IHC) staining was assessed in brain tissue sections derived from cynomolgus monkeys (consecutive with those prepared and stained with H&E). hGH staining of brain tissues from vehicle control monkeys was negative.

hGH staining (minimal to mild) was observed in blood vessels (intravascular, very rare to rare) in the neuropil in 3 HD males and 2 HD females after 52 weeks of dosing. The hGH detected in these locations correlated with mPEG staining and suggests that this staining represents intact lonapegsomatropin within the blood vessels. No other hGH staining was observed in the brain tissues of lonapegsomatropin-dosed monkeys.

The Reviewer considers the HD (4.8 mg hGH/kg/QW) the most clinically relevant NOAEL as the subcutaneous administration of lonapegsomatropin to juvenile cynomolgus monkeys (0.4, 1.6, and 4.8 mg hGH/kg/QW) for 52 weeks followed by a 52-week recovery period resulted in no adverse findings.

7 Genetic Toxicology

Genotoxicity assays were primarily conducted to assess the genotoxic potential of the TransCon linker ((b) (4) attached to mPEG) and the (b) (4) leaving group as the hGH and mPEG components of lonapegsomatropin are not expected to act as genotoxic agents.

A standard battery of in vitro and in vivo genotoxicity assays was conducted (GLP compliant) and included two in vitro studies (Ames assay and mammalian chromosome aberration assay) and one in vivo study (rat bone marrow erythrocyte micronucleus assay). In vitro studies were conducted with partly cleaved lonapegsomatropin to facilitate exposure to both lonapegsomatropin and the products of its autocleavage in the Ames (5000 µg/plate) and ChromAbb (500 µg/mL) assay systems. In vivo genotoxicity was assessed after 5 weeks of dosing at ≤ 3 mg hGH/kg/QW in SD rats.

(b) (4) was not found to be genotoxic during in vitro genotoxicity assessments (using partly cleaved lonapegsomatropin) at concentrations of free (b) (4) up to 2300 ng/mL (Ames) and 637 ng/mL (ChromAbb). In comparison, the mean C_{max} of (b) (4) following 52 weeks of lonapegsomatropin administration in monkeys was 41 pg/mL and (b) (4) levels were generally below the LLOQ (25 pg/mL), following 26 weeks of treatment in GHD pediatric patients (CT-302).

No significant increase in the incidence of micronucleated polychromatic erythrocytes was observed in the bone marrow of male SD rats and lonapegsomatropin (and the products of its autocleavage (TransCon linker and the (b) (4) leaving group) are considered negative for in vivo clastogenicity. The levels of (b) (4) employed in the nonclinical safety assessment (in vitro and in vivo) of genotoxicity greatly exceeded those observed in the clinic and are therefore considered adequately qualified. Comprehensive quantitative structure-activity relationship (QSAR) assessments evaluating (b) (4) and the remainder of the inactive mPEG component of lonapegsomatropin (b) (4) (b) (4) were also performed. Based on these evaluations, it was concluded that the structural elements of (b) (4) and mPEG do not demonstrate evidence of mutagenicity, carcinogenicity, genotoxicity or cardiotoxicity.

Based on the data, the Reviewer concludes that there is a minimal concern regarding the genotoxic potential of lonapegsomatropin, hGH or the products of its autocleavage (mPEG, TransCon linker-mPEG and (b) (4)) in children with GHD.

8 Carcinogenicity

The carcinogenicity assessment of lonapegsomatropin was based on a weight of evidence approach that incorporated nonclinical and clinical data, as well as published nonclinical and clinical information. The carcinogenicity assessment evaluated the potential carcinogenic properties of lonapegsomatropin, the products of its autocleavage (hGH, mPEG, (b) (4)) and a marker of GHR activation (IGF-1) and supported a conclusion that additional rodent carcinogenicity studies would not add value to the

assessment for lonapegsomatropin. The weight of evidence evaluation/determination for lonapegsomatropin was based on the following key points:

1. hGH: There is significant clinical experience with chronic hGH administration, the carcinogenicity profile is known, it is a biotechnology-derived product and hGH is an endogenous protein that will be restored to physiological levels with lonapegsomatropin administration in the proposed clinical population.

2. (b) (4) systemic exposure to this hydrolysis product is below the qualification threshold, it was qualified in a battery of genetic toxicology studies that were negative for genotoxic potential and it is present at extremely low levels and/or below levels of detection in humans.

3. Linker (b) (4) and mPEG40-linker: Both the linker and mPEG40-linker were qualified in a battery of genetic toxicology studies that were negative for genotoxic potential and the linker remains bound to the mPEG40 molecule, of which the linker makes up only (b) (4)% of the entire molecule. Furthermore, evaluation of this component in a carcinogenicity study is not likely to provide added benefit, given that it will be administered with hGH, which is known to have proliferative effects. Based on the overall weight of evidence, including past experiences with PEGylated products in the literature and previous applications, there is no evidence supporting an increased concern for carcinogenicity, particularly in the presence of co-administration with hGH.

4. Lonapegsomatropin and all 3 of the hydrolysis products (released hGH, (b) (4) and mPEG40-linker) were evaluated in a chronic 52-week juvenile monkey toxicology study, wherein the only pre-neoplastic findings that were observed (in mammary tissue) were attributable to the pharmacodynamic activity, and potential exaggerated pharmacological activity, of the hGH portion of lonapegsomatropin.

5. Rats (and presumably rodents in general) develop binding and neutralizing antibodies that have a variable and inconsistent impact on systemic exposure to lonapegsomatropin. Thus, maintaining systemic exposure levels over the duration of a 2-year carcinogenicity study would be unrealistic.

6. Labeling for lonapegsomatropin will include language similar to approved hGH products indicating that patients should be monitored for progression or recurrence of neoplasms and that an increased risk of a second neoplasm exists for childhood cancer survivors treated with hGH.

Due to the known growth promoting effects of hGH, treatment with GH products is contraindicated in the presence of active malignancy. Based on the data, the Reviewer concludes that the carcinogenic risk of lonapegsomatropin in children with GHD would not differ from marketed hGH products.

9 Reproductive and Developmental Toxicology

Reproductive and developmental toxicity studies were conducted in SD rats and included a fertility and early embryonic development study (9001005, GLP) and an embryo-fetal development study (497516, GLP) of lonapegsomatropin. In addition, a pre- and post-natal development toxicity study (495765, GLP) was conducted with ACP-001 (predecessor DP with larger PEG moiety). Despite the presence of anti-drug antibodies (ADAs), SD rats demonstrated exposure to lonapegsomatropin/ACP-001 and were well tolerated. There was no evidence of reproductive toxicity observed in the DART studies conducted in SD rats and the HD was considered the NOAEL for each study.

The NOAEL associated with the fertility and early embryonic development study of Lonapegsomatropin (EOD, 28 days, SC) in rats (9001005) was determined to be 1.4 mg hGH/kg/48 hr (4.9 mg hGH/kg/week) as there was no evidence of effects on reproductive performance/fertility, including ovarian, uterine or male reproductive parameters and there were no embryo-lethal effects at doses ≤ 1.4 mg hGH/kg. The EFD toxicity study of Lonapegsomatropin (GD 6 and 13, SC) in rats (497516) was associated with a maternal and fetal NOAEL of 3 mg hGH/kg/dose.

The pre- and post-natal development study of ACP-001 (Days 6, 13 and 20 of gestation and lactation, SC) in SD rats (495765) was associated with a maternal and fetal NOAEL of 3 mg hGH/kg/dose (HD) and was deemed directly applicable to the reproductive and developmental safety assessment of lonapegsomatropin.

Exposure Margins Between NOAELs in Pregnant Rats and Humans (0.24 mg hGH/kg)

Analyte	Species	C_{max} (ng/mL)[a]	MoE (C_{max})
hGH	Pregnant Rats [b]	86	6
	Humans (Adults) [c]	13	-
Lonapegsomatropin	Pregnant Rats [d]	1160	2
	Humans (Adults) [c]	535	-

[a] $C_{12h/24h}$ in rats are used for comparison to C_{max} as only proof of exposure was obtained in the development and reproductive toxicity studies in rats.
[b] Data from pre- and post-natal development study (495765) in rats dosed with the predecessor product of lonapegsomatropin (ACP-001) at Week 2, 24 hr post-dose at NOAEL of 3 mg hGH/kg/week
[c] Data from Phase 1 clinical trial CT-101 in healthy adults after a SD of lonapegsomatropin at 0.24 mg hGH/kg
[d] Data from EFD study (497516) in rat, at Week 2, 12 h postdose at NOAEL of 3 mg hGH/kg/week
Abbreviation: MoE: Multiple of Exposure

In the rabbit embryo-fetal development study, the administration of lonapegsomatropin elicited maternal toxicity and subsequent intrauterine death and fetal malformations. In addition, neutralizing ADAs were generated in rabbits and resulted in a loss of PD activity. Embryo-fetal findings were considered a consequence of maternal toxicity and a NOAEL could not be determined. Due to the maternal toxicity observed at all dose levels and the presence of neutralizing ADAs, the study was considered uninformative with regards to the evaluation of the potential embryo-fetal developmental toxicity of lonapegsomatropin and the developmental and reproductive toxicology assessment conducted in rats was considered sufficient (FDA Meeting Advice) to support the BLA.

hGH Exposure Levels in Rabbits (LOAEL) Relative to GHD Children

Species	Dose	hGH ($AUC_{0-168hr}$) (ng.hr/mL)	MoE (AUC)
Rabbit	0.35 mg hGH/kg/48 hr)[a]	640	1.4
Human	0.24 mg hGH/kg/week)[b]	453	-

[a]C24hr exposure was comparable between study 9001004 (GLP embryo-fetal study) and study 1704-039 (PK study). Therefore, study 1704-039 was used to estimate an exposure margin between rabbits and humans. AUC_{0-48h} on Day 1 for the 0.35 mg hGH/kg/48 hr dose level was extrapolated to 830 ng.hr/mL, based on data from the 0.7 mg hGH/kg/48 hr dose level using a factor of 3 (AUC_{0-48h} at Day 1: 2490 for 0.7 mg hGH/kg/48 hr). C24h was comparable at Day 1 and 7 and a factor of 3.5 was used to extrapolate AUC_{0-168h} from AUC_{0-48h}. Extrapolated rabbit AUC_{0-168h} = 2900 ng.hr/mL for 0.35 mg hGH/kg/48 h. Accounting for observed cross-reactivity of 450% in the rabbit assay, results in an AUC_{0-168h} of 640 ng.hr/mL.

[b] Human hGH data from the Phase 3 study in GHD children at Week 13 (CT-301). Mean human AUC_{0-168h} = 832 ng.hr/mL. Accounting for observed cross-reactivity in the human hGH assay, results in an AUC_{0-168h} of 453 ng.hr/mL.

Abbreviation: MoE: Multiple of Exposure

Histopathological assessments of tissues following weekly administration (SC) of lonapegsomatropin to SD rats (26-Weeks) and cynomolgus monkeys (52-Weeks), in repeat-dose toxicity studies, yielded no adverse effects on reproductive organs at doses ≤ 4.8 mg hGH/kg/week.

Based on the cumulative DART data, the Reviewer concludes that there is minimal concern regarding the reproductive and development toxicity potential of lonapegsomatropin, hGH or the products of its autocleavage in children with GHD.

10 Special Toxicology Studies

Local Tolerance

Injection site reactions were infrequent, mild and transitory and typical of the SC route of administration. In the 52-week repeat-dose toxicity study in monkeys (1704-035), lonapegsomatropin-related dermal scores were comparable to those observed in the vehicle control group.

Two structural alerts linked to the (b) (4) structure were identified in relation to skin sensitization (DEREK). The skin sensitization potential of (b) (4) was assessed in a mouse local lymph node assay (520487) where partly cleaved ACP-001 (predecessor product) was applied to the dorsum of the ears of mice for 3 consecutive days at concentrations up to the maximum limit of the formulation (25 mg partly cleaved ACP-001/mL (23 µg (b) (4) /mL). The partly cleaved ACP-001 (containing (b) (4)) did not elicit changes indicative of skin sensitization in mice.

Phototoxicity

To investigate the phototoxic potential of (b) (4) an assessment of absorption of light within the 230 to 700 nm spectrum was performed (TW1104121). Consistent with the lack of chromophores in the (b) (4) no significant absorbance was observed, thus, no concern for phototoxicity was demonstrated for (b) (4)

11 Integrated Summary and Safety Evaluation

The nonclinical safety evaluation of lonapegsomatropin and the products of its autocleavage (hGH, (b) (4) and mPEG), included genotoxicity, safety pharmacology, local tolerance, reproductive toxicity and repeat-dose toxicity studies in rats (≤ 26-weeks) and juvenile monkeys (≤ 52 weeks) that did not demonstrate any adverse safety signals and/or effects that would preclude use in the intended GHD clinical population.

The HD (4.8 mg hGH/kg/QW) was considered the most clinically relevant NOAEL in the 26-week chronic toxicity study conducted in SD rats, as subcutaneous administration of lonapegsomatropin (1.2, 2.4, and 4.8 mg hGH/kg/QW) for 27 weeks followed by a 27-week recovery period resulted in no adverse findings.

The results of the 26-week rat study were confounded by the development of both binding and neutralizing anti-drug antibodies (ADAs) in the majority of rats that were detectable by Day 43. These ADAs markedly affected exposure to lonapegsomatropin (and to liberated hGH) and impaired PD activity (IGF-1). Many low-dose (LD) and mid-dose (MD) rats had no measurable level of drug exposure by Day 43. Exposure was maintained in the majority of HD rats until the end of dosing, although the levels were generally reduced compared to the those observed prior to ADA development (Day 1). Lonapegsomatropin was well tolerated and body weight/organ weight changes were, in general, consistent with the pharmacology of human growth hormone (hGH). As exposure and pharmacodynamic activity were maintained at the HD the study was considered informative with regards to the risk assessment of lonapegsomatropin.

In the 52-week chronic toxicity study, the majority of monkeys did not develop ADAs after exposure to lonapegsomatropin (consistent with the greater sequence identity between human and monkey GH). Histopathological findings in the mammary gland (exudate, galactocele, vacuolation, mononuclear cell infiltration and lobular hyperplasia) were observed in monkeys and are consistent with the ability of hGH to stimulate mammary tissue growth directly through an interaction between growth hormone and epithelial prolactin receptors or indirectly by increasing circulating levels of IGF-1.

In patients with acromegaly (a condition characterized by hypersecretion of GH and consequently higher endogenous IGF levels) several studies have demonstrated an increase in the risk of gastro-intestinal cancers while other studies indicate a modest association between higher circulating IGF levels and an increased risk for prostate, breast, colorectal and ovarian cancer[5].

The Reviewer considers the HD (4.8 mg hGH/kg/QW) the most clinically relevant NOAEL as the subcutaneous administration of lonapegsomatropin to juvenile cynomolgus monkeys (0.4, 1.6, and 4.8 mg hGH/kg/QW) for 52 weeks followed by a 52-week recovery period resulted in no adverse findings.

[5] S. John Weroha and Paul Haluska, Endocrinology Metabolism Clin North Am. 2012 Jun; 41(2): 335–350.

Exposure Margins Between SD Rats and Cynomolgus Monkeys at the NOAEL/HD (4.8 mg hGH/kg/QW) and GHD children (0.24 mg hGH/kg/QW) at Steady State

Analyte	Species	Week	$AUC_{0\text{-}168hr}$ (ng.hr/mL)	C_{max} (ng/mL)	MoE	
					AUC	C_{max}
hGH	Rat (Male)[a]	13	N/D	15.9	**-**	**0.9**
	Rat (Female)[a]	13	N/D	84.3	**-**	**4.6**
	Rat (Male)[a]	26	N/D	13.8	**-**	**0.7**
	Rat (Female)[a]	26	N/D	25.7	**-**	**1.4**
	Monkeys[bc]	1	26100	320	**38**	**17**
	GHD Children[d]	13	678[e]	18.5	**-**	**-**
mPEG	Rat (Male)[a]	26	6070000	48500	**3.5**	**3.7**
	Rat (Female)[a]	26	8840000	61000	**5.1**	**4.7**
	Monkeys[b]	26	54800000	391000	**31**	**30**
		52	58900000	425000	**34**	**32**
	GHD Children[d]		1740000	13100	**-**	**-**
Lonapegsomatropin	Rat (Male)[a]	13	N/D	726	**-**	**0.6**
	Rat (Female)[a]	13	N/D	1340	**-**	**1.1**
	Rat (Male)[a]	26	N/D	251	**-**	**0.2**
	Rat (Female)[a]	26	N/D	686	**-**	**0.6**
	Monkeys[b]	26	3960000	42200	**54**	**34**
		52	3850000	37300	**52**	**30**
	GHD Children[d]	13	74000	1230	**-**	**-**

[a] Data from 27-week repeat-dose toxicity study (1704-037) in SD rats, C_{24h} are used for comparison to C_{max} due the incidence of anti-hGH antibodies impacting the accuracy of the TK parameters in the SD rat studies.
[b] Data from 52-week repeat-dose toxicity study (1704-035) in cynomolgus monkeys.
[c] Baseline corrected hGH used for cynomolgus monkey (corrected for baseline cynomolgus monkey GH).
[d] Data from the Phase 3 clinical trial CT-301 in GHD children. PK subset at Week 13
[e] $AUC_{0\text{-}t}$
Abbreviations: MoE: Multiple of Exposure, N/D: No Data

(mPEG Safety Assessment)

A toxicologic evaluation of mPEG derived from the administration of lonapegsomatropin was conducted in adolescent/adult SD rats (≤ 27-weeks) and juvenile cynomolgus monkeys (≤ 52-weeks) where no adverse findings were observed at lonapegsomatropin dose levels ≤ 4.8 mg/kg/week (20-fold above the expected clinical therapeutic dose level of 0.24 mg hGH/kg/week in GHD children).

Immunohistochemical (IHC) staining of mPEG and vacuolation were noted in brain tissues derived from rats and monkeys administered lonapegsomatropin and were predominantly observed in highly vascularized areas associated with the blood-brain barrier and blood-CSF barrier. mPEG stained vacuoles tended to be small in size and the presence of mPEG staining and vacuolation were not associated with a distortion of the cytoplasmic or nuclear compartments, degeneration, necrosis or inflammation. Only partial recovery of mPEG (IHC) staining and vacuolation was evident after the 27- and

52-week recovery period in rats and monkeys, respectively, where mPEG continued to be associated with several cell types and vacuolization of the choroid plexus. Clinical signs of neurotoxicity (e.g. tremors, convulsions, reactivity to handling or unusual behavior) were not observed in either SD rats or cynomolgus monkeys and while neurological function was not specifically assessed in the lonapegsomatropin clinical program, the report concluded that there were no direct or indirect safety signals indicative of neurologic adverse reactions in children with GHD.

The level of mPEG in the CSF did not exceed the LLOQ (500 ng/mL) in either SD rats or cynomolgus monkeys administered lonapegsomatropin and hGH/mPEG were not detected in areas of the brain associated with high levels of growth hormone receptor (GHR) expression, suggesting that hGH receptor-mediated uptake and active transport across the blood-brain/CSF barriers are not involved in the uptake/distribution of mPEG.

The no observed effect level (NOEL) for the presence of H&E stained vacuoles in the brain of cynomolgus monkeys was the LD (0.40 mg hGH/kg/week) where systemic mPEG exposure (3330 ug.hr/mL, AUC) was 2-fold above the clinical mPEG exposure level (0.24 hGH mg/kg/Week, 1740 ug.hr/mL, AUC) in GHD children. The vacuolation observed by H&E staining in the choroid plexus epithelial cells persisted in MD/HD recovery monkeys (slow elimination) while macrophage vacuolation tended to resolve.

The mPEG dose level (total mPEG load) administered to children with GHD was 8-fold below the theoretical threshold (3.7 mg PEG (40 kDa)/kg/week or 0.4 μmol/kg/month) recommended by the Committee for Medicinal Products for Human Use (CHMP 2012) for choroid plexus epithelial cells vacuolation. In addition, systemic mPEG concentrations in children with GHD at steady state (15 μg/mL) were 7-fold below the mPEG exposure/vacuolation threshold (100 μg/mL) defined by Jacobsen/Bjørnsdottir (FDA Briefing Document and BioSafe EU, 2017).

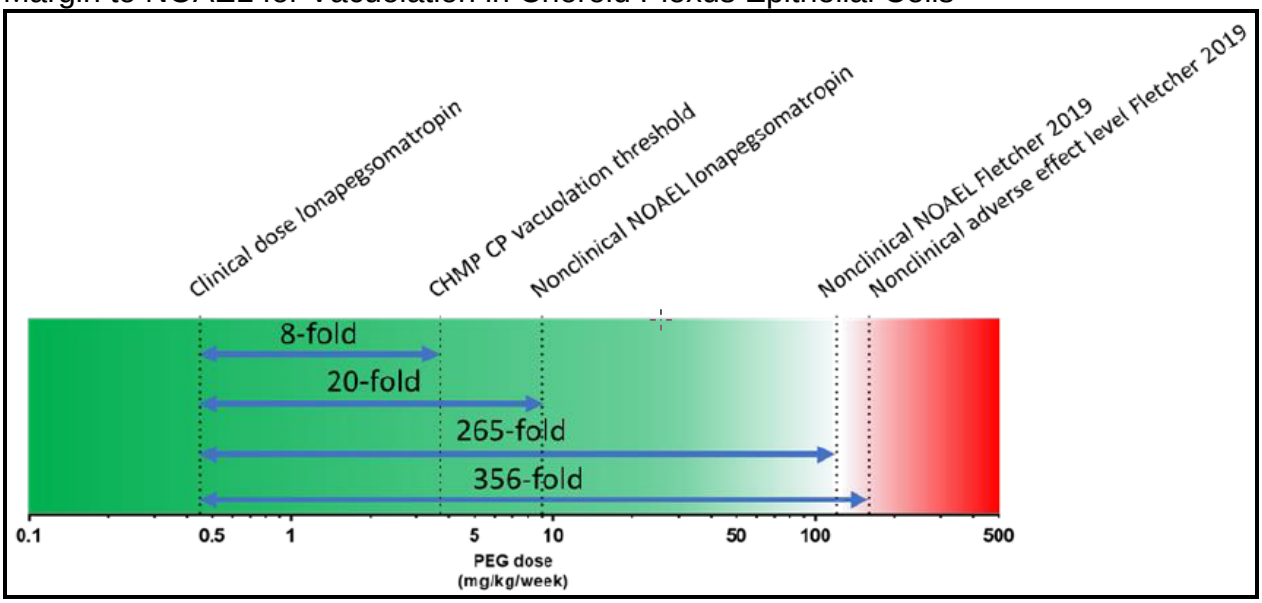
Adverse findings in the choroid plexus have been described previously (Fletcher 2019) in cynomolgus monkeys administered a PEGylated compound for 3 months.[6] In this study, a 160 mg PEG/kg/QW dose (LOAEL) was well tolerated by cynomolgus monkeys but elicited adverse histologic perturbations of the tissue architecture in the choroid plexus. Adverse histopathological findings were absent at doses ≤ 120 mg PEG/kg/week (NOAEL), establishing a threshold for PEG associated adverse changes in the choroid plexus at the 3-month timepoint in cynomolgus monkeys.

The 160 mg PEG/kg/QW dose (LOAEL) from the Fletcher paper represents a dose that is 350-fold higher than the mPEG dose of lonapegsomatropin at the therapeutic dose level of 0.24 mg hGH/kg/week (0.45 mg mPEG/kg/week). In addition, the therapeutic

[6] Fletcher AM, Tellier P, Douville J, et al. Adverse vacuolation in multiple tissues in cynomolgus monkeys following repeat-dose administration of a PEGylated protein. Toxicol Lett. 2019;317:120-129.

dose level of lonapegsomatropin provides a dose margin of 265-fold to the NOAEL at 120 mg PEG/kg/week defined by Fletcher 2019 (See Sponsor's Figure Below).

Lonapegsomatropin mPEG Dose Margin to CHMP Vacuolation Threshold and Dose Margin to NOAEL for Vacuolation in Choroid Plexus Epithelial Cells



Source: Ascendis Pharma - Response to Information Request (December 4th 2020)

Taken together and based on the assumption that the nonclinical pharmacokinetic data, H&E vacuolation data and immunohistochemical mPEG staining data are clinically relevant, the therapeutic dose of lonapegsomatropin (0.24 mg hGH/kg/week) is not expected to produce levels of vacuolization in the brain/choroid plexus of GHD children to a degree that would impact cellular, tissue or organ function.

The Reviewer agrees with the Sponsor, that based on the nonclinical safety evaluation, the potential risk for adverse CNS effects related to mPEG induced vacuolation in choroid plexus epithelial cells in the clinical setting is low for lonapegsomatropin at the proposed therapeutic dose level (0.24 mg hGH/kg/week).

(Division of Neurology 1 (DN1) - Nonclinical Consult)

In an independent review conducted by Dr. David Hawver (Division of Neurology), the nonclinical reviewer indicated that, the results of the three pivotal chronic toxicity studies provided compelling evidence that the accumulation of mPEG in the choroid plexus and other brain regions was not adverse at the doses evaluated, which, in the monkey, provided large exposure margins relative to the proposed clinical dose of 0.24 mg hGH/kg/week.

In addition, the reviewer indicated that, the data provided by the Sponsor were consistent with the view of the Society of Toxicologic Pathology (STP) Working Group publication (Irizarry et al. 2018) that accumulation of mPEG in the choroid plexus epithelium of animals administered repeat-doses of PEGylated products appears to be

an adaptive, non-adverse finding, based on the lack of any signs of degeneration or dysfunction in multiple published studies.

The reviewer further cited that there was no evidence of swelling, disruption, or scattering of choroid plexus epithelial cells, or of cuffing of blood vessels by vacuolated macrophages indicative of changes in tissue function in the pivotal chronic toxicity studies of lonapegsomatropin.

The reviewer concluded that, the available evidence provided in the pivotal toxicity studies is consistent with a low risk at the proposed human dose. While the studies did not include rigorous neurological testing, neurobehavioral assessments or analysis of CSF composition, based on the relatively large exposure margins achieved in the monkey and the lack of evidence that minimal to moderate accumulation of mPEG is adverse in the absence of any other abnormalities, additional nonclinical studies may not be needed.

The Division of Pharmacology and Toxicology in the Office of Cardiology, Hematology, Endocrinology and Nephrology (PT-OCHEN) agrees with the nonclinical assessment conducted by the Division of Neurology and concludes that based on the independent analyzes, there are no deficiencies in the nonclinical data that would preclude approval of BLA 761177, and no additional nonclinical studies are recommended to support the safety evaluation of lonapegsomatropin use in GHD children.

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/s/

JEFFREY A QUINN
03/01/2021 03:51:46 PM

TODD M BOURCIER
03/01/2021 05:11:33 PM
I concur with the data evaluation and the regulatory recommendations in Dr. Quinn's review.

Consultation for the Division of General Endocrinology
Evaluation of Pharmacology/Toxicology Data

Requested By:	Sejal Kiani/OCHEN/DGE
Date of Request:	November 14, 2020
Date Received by Reviewer:	December 11, 2020
Desired Completion Date:	January 14, 2021
BLA Number:	761177
Submission Date:	June 25, 2020
Sponsor:	Ascendis Pharma Endocrinology Division, A/S
Reviewer:	David B. Hawver, Ph.D.
Division:	DPT-N
Acting Division Director:	Lois M. Freed, Ph.D.
Deputy ON Director:	Eric Bastings, M.D.
Consult Tech:	Ashley Lewis
Review completion date:	January 19, 2021

Drug Substance:
Lonapegsomatropin (ACP-011; TransCon-linked methoxypolyethylene glycol [4 x 10 kDa] recombinant human growth hormone [rhGH]; TransCon hGH)

Figure 1: Lonapegsomatropin Structure

(BLA 761177 Introduction, page 1)

Drug Product:
Lonapegsomatropin is supplied as a single use, dual-chamber glass cartridge containing sterile, lyophilized powder composed of drug substance (3.0, 3.6, 4.3, 5.2, 6.3, 7.6, 9.1, 11.0, or 13.3 mg, based on hGH content), succinic acid, trehalose dihydrate, and tromethamine (b) (4) and diluent (Water for Injection) in the other chamber, for weekly subcutaneous injection using the recommended Auto-Injector device, after reconstitution.

Pharmacologic class: Human Growth Hormone
Intended clinical population: Children with growth failure due to growth hormone deficiency

Route of administration: SC injection

Reason for Consult:
"DGE is requesting evaluation of the potential risk associated with accumulation of mPEG in the brain including the type of known and/or expected adverse events associated with vacuolation of epithelial cells within the choroid plexus and/or mPEG accumulation in the brain; and whether the pediatric population is at increased risk for the neurologic deficits, etc."

Scope of Review:
This review focused on the three pivotal GLP chronic toxicity studies of SC lonapegsomatropin (a 26-week toxicity study in rat and 26- and 52-week studies in monkey) submitted to BLA 761177 and two recent publications relevant to the issue.

ACP-011: A 26-Week Subcutaneous Repeated-dose Toxicity Study in Rats Followed by a 26-Week Recovery Period ([redacted] Study 1704-037; GLP)

Sprague Dawley rats (N=15/sex/group main study; 6/sex/group 27-week recovery; 9/sex/group TK; 7 weeks old at initiation of dosing) were administered lonapegsomatropin (0, 1.2, 2.4, 4.8 mg hGH/kg SC) once weekly for 27 weeks. The Neuropathology report of the H&E stained brain sections contained only scanned images from the slides from one representative animal and a record of the presence of anatomical structures on each slide, perhaps because the data evaluated in the 26-week study of lonapegsomatropin in monkey suggested that IHC staining was generally more sensitive than H&E staining for identification of vacuoles in choroid plexus epithelium.

Immunohistochemistry (IHC) analysis of brain sections revealed minimal to mild mPEG staining in the cytoplasm of choroid plexus epithelial cells in fine-bore granules (17/30 LD, 29/30 MD, 28/28 HD main study; all recovery animals) and, less often, small vacuoles (3/30 LD, 15/30 MD, 22/28 HD main study; 8/12 LD, 11/11 MD, 11/11 HD recovery). The frequency of positive cells per section generally varied from <1% to 1-5% in LD, 1-5% to 6-25% in MD, and up to 26-50% in some HD animals. The incidence and frequency of staining increased during the 27-week recovery period, suggesting that continued uptake into choroid plexus occurred as mPEG was cleared from other tissues.

Minimal to mild cytoplasmic mPEG staining in fine granules was also observed in specialized glial cells, ependymal cells, and endothelium of main study and/or recovery animals, although the frequency was much lower than in choroid plexus epithelial cells. No staining of interstitial macrophages in choroid plexus or other brain regions was observed.

Moderate or marked staining of mPEG was not observed in any brain sections of any animal at any dose. The consensus view of the IHC study Principal Investigator (James, Raymond, DVM, MS, DACVP, [redacted]) and Peer Review Pathologist [redacted] was that there was “no evidence of distortion of cytoplasmic or nuclear compartments, degeneration, or necrosis in the choroid plexus epithelial cells with mPEG-stained granules or vacuoles” in main study or recovery animals *(pages 2546 and 2548-2549 of Study Report)*.

The level of mPEG40 in CSF was below the lower limit of quantification (500 ng/mL) in all evaluable samples taken from main study (38 CON, 20 LD, 17 MD, and 19 HD) and recovery (1 CON, 1 MD) animals at termination.

No adverse effects were reported in detailed clinical examinations conducted weekly during the dosing period (at ~24 hours postdose) and during the recovery period. “The observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects

including tremors, convulsions, reactivity to handling, and unusual behavior" *(page 21 of Study Report)*. No additional neurological or neurobehavioral assessments were conducted.

Based on the molecular weights of the hGH (22.125 kDa) and mPEG-Linker (41.5 kDa) components of lonapegsomatropin, administration of the test article at doses of 1.2, 2.4, and 4.8 mg hGH/kg/week corresponded to mPEG doses of approximately 2.25, 4.5, and 9.0 mg mPEG/kg/week, and resulted in steady-state mean mPEG serum exposures (Day 176 $AUC_{0\text{-}168\ hr}$ in M/F) of 1890/2510, 3070/5210, and 6070/8840 µg*hr/mL, respectively. Mean mPEG $AUC_{0\text{-}168\ hr}$ in HD animals on Day 176 were not increased compared to those on Day 85, suggesting serum mPEG was at steady state by Day 85.

ACP-011: A 26-Week Subcutaneous Repeated Dose Toxicity Study in Juvenile Monkeys with a 26-Week Recovery Period ((b) (4) Study 1704-022; GLP)

Juvenile cynomolgus monkeys (N=4/sex/group main study; 2/sex/group 26-week recovery; 15 to 16 months old at initiation of dosing) were administered lonapegsomatropin (0, 1.2, 2.4, 4.8 mg hGH/kg SC) once weekly for 26 weeks. In H&E stained sections of brain evaluated by the Principal Neuropathologist (b) (4) (b) (4) the only test article-related effects observed were the presence of small vacuoles in epithelial cells in the choroid plexus (7/8 HD main study; 2/4 MD and 4/4 HD recovery) and in the resident macrophages within the choroid plexus (4/8 HD main study; 1/4 HD recovery). These effects were mild (Grade 1) and able to be distinguished from extensive artifactual vacuolation only with careful inspection. The Principal Neuropathologist provided the following comments:

- "The distribution of vacuolation (Main Study Group 3 males and females could not be reliably differentiated from the controls whereas the Recovery Group 3 males could be reliably differentiated from the controls) did not in any way indicate a worsening of this very subtle change during the recovery period since in all affected dose groups at both sacrifices, any difference from controls was exceedingly difficult to detect (on the H&E stained sections). There were no detectable differences between the choroid plexus of the Main Study and Recovery sacrifice animals which indicated any exacerbation of the vacuolation."

- "The vacuolation interpreted to be related to the test article was consistently estimated to affect less than 1% of the choroid plexus epithelium and was unlikely to produce any structural or functional impairment."

- "There was no evidence of necrosis of choroid plexus epithelium."

- "Based strictly on the findings from the morphologic evaluation of the H&E-stained sections from Main Study animals, and taking into account the very limited extent of vacuolation and the lack of any choroid plexus epithelium necrosis or other brain findings, it is the recommendation of the (b) (4) PI to the

study director that the morphologic findings in the choroid plexus be considered non-adverse."

IHC analysis of brain sections revealed mPEG staining in the cytoplasm of choroid plexus epithelial cells in fine-bore granules (7/8 LD, 8/8 MD, 7/8 HD main study; all recovery animals) and, less often, small vacuoles (4/8 MD, 7/8 HD main study; 1/4 LD, 4/4 MD, 4/4 HD recovery). Staining was dose-dependent and minimal to moderate. The frequency of positive cells per section generally varied from <1% to 6-25%.

Finely granular and/or vacuolar mPEG staining was also observed in interstitial macrophages within the choroid plexus (granular: 2/8 LD, 6/8 MD, 7/8 HD main study, 1/4 LD, 2/4 MD, 4/4 HD recovery; vacuolar: 1/8 LD, 5/8 MD, 7/8 HD main study, 1/4 LD, 2/4 MD, 4/4 HD recovery). Staining was dose-dependent and minimal to moderate. Usually one to a few macrophages were stained per section.

Cytoplasmic mPEG staining in fine granules and/or vacuoles was also observed in specialized glial cells, ependymal cells, and endothelium of main study animals, although the frequency was much lower than in choroid plexus epithelial cells.

Marked staining of mPEG was not observed in any brain section of any animal at any dose. Comparison of intensity and frequency of mPEG staining in recovery and main study groups was consistent with partial (and slow) reversibility of the granular and vacuolar changes in choroid plexus epithelium and macrophages. The consensus view of the IHC study Principal Investigator (Jennifer L. Rojko, DVM, PhD, DACVP, (b) (4) (b) (4)) and Peer Review Pathologist (b) (4) (b) (4) was that there was "no evidence of distortion of cytoplasmic or nuclear compartments, degeneration, or necrosis" in the choroid plexus epithelial cells containing mPEG-stained granules or vacuoles in main study or recovery animals" *(page 4008 of Study Report)*.

The level of mPEG40 in CSF was below the lower limit of quantification (500 ng/mL) in all evaluable samples taken from main study (4 CON, 6 LD, 8 MD, and 4 HD) and recovery (2 CON, 1 LD, 4 MD, and 4 HD) animals at termination.

No adverse effects were reported in detailed clinical examinations conducted weekly during the dosing period (at ~24 hours postdose) and during the recovery period. "The observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, and unusual behavior" *(page 21 of Study Report)*. No additional neurological or neurobehavioral assessments were conducted.

Based on the molecular weights of the hGH and mPEG-Linker components of lonapegsomatropin, administration of the test article at doses of 1.2, 2.4, and 4.8 mg hGH/kg/week corresponded to mPEG doses of approximately 2.25, 4.5, and 9.0 mg

mPEG/kg/week, and resulted in steady-state mean mPEG serum exposures (Day 176 $AUC_{0\text{-}168\ hr}$) of 12700, 30800, and 60900 µg*hr/mL, respectively (M and F means were combined because there were no consistent sex differences). Mean mPEG $AUC_{0\text{-}168\ hr}$ in HD animals on Day 176 were only increased 13% compared to those on Day 85, suggesting serum mPEG exposures were at steady state by Day 176.

ACP-011: A 52-Week Subcutaneous Repeated Dose Toxicity Study in Juvenile Monkeys with a 52-Week Recovery Period ([redacted] (b) (4) 1704-035; GLP)

Juvenile cynomolgus monkeys (N=4/sex/group main study; 2/sex/group 52-week recovery; 13.5 to 17.5 months old at initiation of dosing) were administered lonapegsomatropin (0, 0.4, 1.6, 4.8 mg hGH/kg SC) once weekly for 52 weeks. In H&E stained sections of brain, the only test article-related effects observed were vacuolation in epithelial cells in the choroid plexus (minimal to mild) and in the resident macrophages within the choroid plexus (minimal) at the MD and HD. The study pathologist [redacted] (b) (4) noted that less than 10% of choroid plexus epithelial cells and few macrophages were affected, that vacuolation in these cell types were "typical of 40 kDa PEGylated compounds" (*Ivins IA, et al., Toxicol Pathol 2015 43(7):959-983*), and that, in the absence of necrosis or degeneration, such findings were "considered unlikely to produce any structural and functional impairment and would not be expected to be of any biologic significance" *(page 3615 of Study Report 1704-035)*. As in the 26-week study in monkey, the accumulation of mPEG in these tissues was much better visualized using ICH.

IHC analysis of brain sections revealed dose-dependent mPEG staining in fine-bore granules (generally <1 µm in diameter) and vacuoles (generally 1-3 µm in diameter) in the cytoplasm of choroid plexus epithelial cells in all animals and in choroid plexus macrophages in all HD main study animals. The incidence, frequency (percentage of positive cells per section), and intensity (minimal, mild, moderate, or marked) of mPEG staining is shown in the table below:

Immunohistochemistry Analysis of mPEG in Brain Sections

	0.4 mg hGH/kg/week (Low Dose)	1.6 mg hGH/kg/week (Mid Dose)	4.8 mg hGH/kg/week (High Dose)
Choroid plexus epithelium Main Study	2/7 1-5% min-mild 5/7 6-25% min-mild	8/8 >75% min-mod	8/8 >75% min-mod
Choroid plexus epithelium Recovery	2/4 <1% min 1/4 1-5% min-mild 1/4 6-25% min-mod	1/4 6-25% min-mod 2/4 26-50% min-mod 1/4 51-75% min-mod	4/4 >75% min-mod
Choroid plexus macrophages Main Study	1/7 one cell mild	1/8 <1% min-mild	3/8 1-5% min 1/8 1-5% min-mild 2/8 1-5% min-mod 2/8 6-25% min-mod
Choroid plexus macrophages Recovery			1/4 1-5% min-mild

The similarity of the mPEG staining in choroid plexus epithelium in MD and HD groups suggests that mPEG levels tissue levels were at steady state. The reduced frequency of positive staining in choroid plexus at the LD and MD after 52 weeks of recovery suggests that clearance of the accumulated mPEG does occur, although at a very slow rate. Clearance of the less severe and/or less frequent mPEG in choroid plexus macrophages was almost complete by the end of the 52-week recovery period.

Cytoplasmic mPEG staining in fine granules and/or vacuoles was also observed in specialized glial cells, ependymal cells, endothelium, and neurons of main study animals, though the incidence, intensity, and frequency was much lower than in choroid plexus epithelial cells. Staining in neurons was of minimal intensity and limited to 5 cells in the hypothalamic region adjacent to the third ventricle in one section from a single HD animal.

Marked staining of mPEG was not observed in any brain sections of any animal at any dose. The consensus view of the IHC study Principal Investigator (James, Raymond, DVM, MS, DACVP, (b) (4)) and Peer Review Pathologist (b) (4) (b) (4) was that there was "no evidence of distortion of cytoplasmic or nuclear compartments, degeneration, or necrosis in any of the mPEG-stained brain elements" *(page 3901 of Study Report)*.

A review of the IHC findings by a Consulting Neuropathologist (b) (4) (b) (4) March 24, 2020) included the following comments:

- "Structure and function of the choroid plexus in humans (and presumably also in monkeys) is generally considered to be present from the earliest stages of brain

development (Liddelow, 2015)." *(Liddelow SA, Frontiers in Neuroscience, March 3, 2015, pp 1-13)*

- In regard to mPEG staining reported in 5 neurons in one HDM, "…it is possible that the staining of the cytoplasm in these neurons may represent lipofuscin instead of mPEG…The biologic significance of finding mPEG within the cytoplasm of a number of hypothalamic neurons in one high dose group male monkey is uncertain, but these stained neurons were otherwise normal in appearance (within the two received micrographs)."

- "The author of this report has had the opportunity to evaluate other preclinical studies performed on PEGylated test articles (in both rodents and non-human primates). In the majority of these studies, with few exceptions, only H&E-stained sections were available, with no immunostaining having been performed. Based on the photomicrographs present in the report on this 52-week study on TransCon hGH (ACP-011), the choroid plexuses in the monkeys were characterized by lesser degrees of choroid plexus vacuolation and by smaller (and fewer) macrophage infiltrates than this pathologist has seen in a number of other studies."

- "While the biologic significance of intracytoplasmic accumulations of mPEG within various cell types is still uncertain, it is concluded by this pathologist (based on the micrographs in the IHC report for Study no. 1704-035) that the presence of mPEG in selected brain regions/cell types was not associated with evidence of cell degeneration or of cell malfunction at the light microscopic level."

The level of mPEG40 in CSF was below the lower limit of quantification (500 ng/mL) in all evaluable samples taken from main study (5 CON, 4 LD, 6 MD, and 6 HD) and recovery (1 CON, 2 LD, 2 MD, and 1 HD) animals at termination.

No adverse effects were reported in detailed clinical examinations conducted weekly during the dosing period (at ~24 hours postdose) and during the recovery period. "The observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, and unusual behavior" *(page 21 of Study Report)*. No additional neurological or neurobehavioral assessments were conducted.

Based on the molecular weights of the hGH and mPEG-Linker components of lonapegsomatropin, administration of the test article at doses of 0.4, 1.6, and 4.8 mg hGH/kg/week corresponded to mPEG doses of approximately 0.75, 3.0, and 9.0 mg mPEG/kg/week, and resulted in steady-state mean mPEG serum exposures (Day 358 $AUC_{0\text{-}168\ hr}$) of 3330, 17600, and 58900 µg*hr/mL, respectively (M and F means were combined because there were no consistent sex differences). Mean mPEG $AUC_{0\text{-}168\ hr}$

in HD animals on Day 358 were only increased 7% compared to those on Day 176, suggesting serum mPEG exposures were at or near steady state by Day 176.

Irizarry et al., 2018, *Toxicologic Pathology* 46(6):616-635

In an article published by the Society of Toxicologic Pathology (STP) Working Group commissioned by the Scientific and Regulatory Policy Committee (SRPC) of the Society of Toxicologic Pathology, Irizarry et al. (2018) provided the following comments on the potential toxicity of PEG accumulation in tissues:

- "In general, PEG-associated cytoplasmic vacuolation has been considered an adaptive, nonadverse finding. This interpretation is supported by the lack of microscopic findings and/or changes in circulating biomarkers indicative of degeneration or dysfunction in affected cells and tissues from many nonclinical toxicity studies of PEGylated biopharmaceuticals..."

- "Regulators and others have expressed hypothetical concerns regarding potential long-term risks associated with PEG exposure and related vacuolation in certain vital tissues/structures such as central nervous system neurons, circumventricular organs, or the choroid plexus…the short-term safety of PEG has been studied extensively without identification of toxicity beyond reports of renal tubular cell vacuolation and degeneration at very high dose levels ..."

- "In some instances, the severity of vacuolation has been marked or severe, occasionally leading to tissue distortion, but yet without demonstrated adverse functional outcomes ... Extended recovery periods typically lead to complete or at least partial reversal depending on the tissues/organs, the degree of which likely reflects the basal turnover rate for cells within a given organ..."

- "Understanding the pathogenesis of PEG-associated findings, the relationship of these findings to tissue distribution of the PEGylated biopharmaceutical or the PEG moiety, and the implications of risk to humans continue to be areas of concern to biopharmaceutical scientists and DRAs. Unfortunately, a comprehensive understanding of the nature and pathogenesis of PEG-associated vacuolation will require further study."

Fletcher et al., 2019, *Toxicology Letters* 317:120-129

Fletcher et al. (2019) described studies of a PEGylated compound administered SC to cynomolgus monkeys at doses equivalent to up to 120 and 160 mg PEG/kg/week for 3 months. H&E staining showed dose-dependent increases in vacuolation in macrophages in several tissues (choroid plexus, pituitary gland, kidney, and choroid of the eye), as well as in epithelial cells in the choroid plexus and renal tubules. At doses ≤ 120 mg PEG/kg/week, there were no morphologic changes other than the vacuolation. However, at 160 mg PEG/kg/week, potentially adverse effects were observed in the choroid plexus, pituitary, renal tubular cells, and choroid of the eye. In the choroid

plexus, blood vessels were surrounded by clusters of vacuolated macrophages, which may interfere with transport between blood and CSF. Similarly, in the pituitary gland, cuffs of vacuolated macrophages surrounded blood vessels at the margin of the pars nervosa and intermedia, which could interfere with pituitary function. In renal cortical tubules, epithelial cells were swollen, obliterating the tubular lumen, or disrupted and scattered. Finally, in the choroid of the eye, minimal to moderate vacuolation of macrophages was associated with minimal edema of the choroid and reduction of the local density of melanin, which could adversely affect the blood supply to the outer retina and/or the anti-reflective properties of the choroid, respectively. IHC staining for PEG confirmed that the vacuolation in choroid plexus and kidneys was associated with cytoplasmic PEG. Thus, it appears that the accumulation of PEG with repeated administration of sufficiently high doses of PEGylated products may result in impairment of function caused by physical disruption of epithelium or swarms of vacuolated macrophages surrounding blood vessels and/or crowding out other cells.

Summary and Discussion

In the pivotal 26-week toxicity study in rat, administration of lonapegsomatropin (0, 1.2, 2.4, 4.8 mg hGH/kg/week SC) resulted in dose-dependent increases in the incidence, intensity, and frequency of mPEG in cytoplasmic granules and, to a lesser extent, vacuoles, in choroid plexus epithelium. The intensity varied from minimal to mild and the frequency varied from <1% of cells per section to 26-50%. The incidence and frequency of mPEG staining increased during the 27-week recovery period, although remained minimal to moderate and in ≤50% of cells. Minimal to mild mPEG staining in fine granules in specialized glial cells, ependymal cells, and endothelium was observed at lower frequency than that in choroid plexus epithelium. No staining of mPEG in interstitial macrophages was observed in brain sections. Except for the accumulation of granular and vacuolar mPEG, brain sections appeared morphologically normal. There were no adverse clinical signs observed in weekly detailed examinations. mPEG40 was undetectable in CSF samples from main study or recovery animals. The NOAEL was the HD (4.8 mg hGH/kg/week).

In the pivotal 26-week toxicity study in juvenile monkey, administration of lonapegsomatropin (0, 1.2, 2.4, 4.8 mg hGH/kg/week SC) resulted in mild vacuolation of choroid plexus epithelium (<1% of cells) and resident macrophages in H&E stained brain sections from HD animals. Similar changes were observed in MD and HD recovery animals. In the absence of any other morphological findings in brain, these effects were considered non-adverse by the study neuropathologist. IHC staining for mPEG showed much more clearly than H&E staining that minimal to moderate mPEG accumulation in the choroid plexus epithelium occurred in almost all animals at all doses, in the form of find-bore granules and, less often, small vacuoles affecting <1% to 6-25% of cells per section, and in one or two resident macrophages per section. The frequency and intensity of staining were slightly reduced after the 26-week recovery period, suggesting mPEG is cleared very slowly from these tissues. Cytoplasmic granular and/or vacuolar staining of mPEG in specialized glial cells, ependymal cells, and endothelium was observed at lower frequency than in choroid plexus epithelium.

There were no adverse changes in mPEG-stained brain sections or in clinical signs observed in weekly detailed examinations. mPEG40 was undetectable in CSF samples from main study or recovery animals. The NOAEL was the HD (4.8 mg hGH/kg/week).

In the pivotal 52-week toxicity study in juvenile monkey, administration of lonapegsomatropin (0, 0.4, 1.6, 4.8 mg hGH/kg/week SC) resulted in the accumulation of mPEG in the cytoplasm of choroid plexus epithelium as visualized using IHC staining of brain sections. mPEG-stained fine-bore granules and small vacuoles were present in ≤25% of cells at the LD (minimal to mild) and >75% of cells at the MD and HD (minimal to moderate). LD and MD animals showed partial reversibility after the recovery period. Cytoplasmic mPEG staining in choroid plexus macrophages, specialized glial cells, ependymal cells, and endothelium were generally observed at lower incidence, severity, and frequency compared with choroid plexus epithelium. The Consulting Neuropathologist concluded that there was no evidence of cell degeneration or cell malfunction associated with the accumulation of mPEG in the brain regions examined. There were no adverse clinical signs observed in weekly detailed examinations. mPEG40 was undetectable in CSF samples from main study or recovery animals. The NOAEL was the HD (4.8 mg hGH/kg/week).

As shown in the table below, the NOAELs in the 26-week study in rat, the 26-week study in monkey, and the 52-week study in monkey were associated with serum mPEG exposure margins of 3.5x, 35x, and 34x, respectively, compared to exposures in children at the proposed clinical dose of 0.24 mg hGH/kg/week. The lower margin in rat is not necessarily cause for concern, as the intensity and frequency of choroid plexus epithelial mPEG staining in HD rats (minimal to mild and up to 26-50%) was lower than that in the 52-week monkey study (minimal to moderate and all >75%), perhaps indicating that higher mPEG exposures would have been tolerated in rat.

mPEG Serum Exposure Margins

	NOAEL (mg hGH/kg/week)	**NOAEL (mg PEG/kg/week)**	**$AUC_{0\text{-}168\ hr}$ (µg*hr/mL)**	**Exposure Margin**
26-Week Rat	4.8	9.0	6070	3.5x
26-Week Juvenile Monkey	4.8	9.0	60900[#]	35x
52-Week Juvenile Monkey	4.8	9.0	58900*	34x
Pediatric Human Subjects (3-12 years old)	0.24	0.45	1740[&]	--

[&]Week 13 data, Study CT-301 in Prepubertal Children with Growth Hormone Deficiency
*Day 358 data, Study 1704-035, Mean of M & F
[#]Day 176 data, Study 1704-022, Mean of M & F
[$]Day 176 data, Study 1704-037, Mean of M

Considered together, the results of the three pivotal chronic toxicity studies provide compelling evidence that the accumulation of mPEG in the choroid plexus and other brain regions was not adverse at the doses evaluated, which, in monkey, provided large exposure margins relative to the proposed clinical dose of 0.24 mg hGH/kg/week.

The data provided by the sponsor are consistent with the view of the STP Working Group publication (Irizarry et al. 2018) that accumulation of mPEG in the choroid plexus epithelium of animals administered repeated doses of PEGylated products appears to be an adaptive, non-adverse finding, based on the lack of any signs of degeneration or dysfunction in multiple published studies.

The studies reported by Fletcher et al. (2019) demonstrated that repeated administration of a PEGylated product at high enough doses can lead to accumulation of so much PEG that cells become swollen and/or disrupted (e.g., renal tubular epithelium) and/or excessive numbers of vacuolated macrophages interfere with tissue function (e.g., choroid plexus, pituitary gland, and choroid of the eye) and suggest that abnormalities in these areas might be expected at excessive doses of PEGylated products. There was no evidence of swelling, disruption, or scattering of choroid plexus epithelial cells, or of cuffing of blood vessels by vacuolated macrophages in the pivotal chronic toxicity studies of lonapegsomatropin provided in BLA 761177.

The question of whether the pediatric population may be at increased risk of neurologic deficits secondary to the accumulation of PEG in the brain remains open. There were no indications of neurological impairment in the rats or juvenile monkeys used in the pivotal studies (approximately equivalent to a human 12-year old or 5- to 6-year old, respectively, at the start of dosing). However, while these studies included extensive neurohistopathology evaluations, they lacked neurobehavioral assessments that are typically included in a juvenile animal toxicology study. The sponsor of BLA 761177 argued that PEG accumulation is not likely to affect the development of the choroid plexus because that takes place in humans prior to the period of exposure; however, that does not preclude an adverse effect on CSF production having a secondary effect on development of other brain regions. The absence of PEG in CSF samples analyzed in the pivotal studies suggests that the blood:CSF barrier had not been compromised, although detailed investigations on the potential effects of PEG accumulation on CSF composition have not yet been conducted.

The available evidence provided in the pivotal toxicity studies is consistent with a low risk at the proposed human dose. While the studies did not include rigorous neurological testing, neurobehavioral assessments, or analysis of CSF composition, based on the relatively large exposure margins achieved in monkey and the lack of evidence that minimal to moderate accumulation of mPEG is adverse in the absence of any other abnormalities, additional nonclinical studies may not be needed.

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